Printed in Switzerland by Zollikofer & Co. Inc., St.Gall

TABLE OF CONTENTS

PREFACE

In our department, a center for rheumatic and bone diseases, we had the occasion to examine and study patients with industrial fluorosis. We already knew from our dentist colleagues about the usefulness of small doses of fluorides in the prophylaxis of dental caries, and we were aware that clinical trials were underway trying medium doses of fluorides in the treatment of osteoporosis. Our fluorotic patients who had ingested too high doses of fluorides demonstrated the important bone changes fluorides can induce. To be able to use fluorides for stimulation of a deficient bone would be a major step forward in the treatment of such a frequent disease, generalized osteoporosis.

The moment seemed opportune to gather together the experts in various fields of fluoride research and to discuss with them different aspects in order to evaluate the fluoride induced bone changes and their possible benefit for osteoporotic patients.

A symposium was organized with the precious collaboration of Dr. W. M. ZINN, chairman of the Swiss Society of Physical Medicine and Rheumatology, Professor B COURVOISIER, La Chaux-de-Fonds, and Dr. T. L. VISCHER, formerly from our department. It took place in a relaxed atmosphere at the cheerful spa Bad Ragaz, Switzerland, on April 17th to 19th, 1969. The results are definitely useful. Issues on the mode of action of fluorides were clarified, the toxic effects were evaluated, and the therapeutic trials presented showed encouraging results.

The success of this meeting is due to the participants who brought with them their knowledge and interest in this field. The material assistance pro-

Osteoporosis or diffuse bony atrophy is a common disease, characterized best by a loss of bone mass. Little is really known about its pathogenesis and etiology and, therefore, no specific treatment is available. Until now, most drugs proposed for treatment have proved unsatisfactory. On X-ray films, osteoporotic bone looks very radiolucent. The opposite was observed in cases of skeletal fluorosis, where one of the characteristics is increased radioopacity of the skeleton. This drew the attention to fluorides as possible therapeutic agents in osteoporosis.

Extensive work has been done during the last years on the biological effects of fluoride. Fluoride ions are taken up almost exclusively by the bones, where they exert their effect. In a medium dose, fluorides seem to stimulate bone formation, induce somewhat increased resorption and cause changes in the degree of mineralization. Since the stimulating activity prevails over bone resorption, as will be shown in this volume, the net result is an increase in bone mass. Thus, a favourable action of fluorides in the treatment of osteoporosis seems possible.

Investigators in the field of fluoride represent widely different areas such as biochemistry, public health, dentistry, anatomy, veterinary medicine, internal medicine, etc. Many data are accumulating and the need for assembling them becomes urgent. The present volume attempts to give an up-to-date account on fluorides in view of their possible use in the treatment of osteoporosis.

The first section is devoted to basic facts about fluorides, including their influence on enzymes, and two reports of their action on the bones of laboratory animals. New insights not only arise from the experimental data but also from the thoughts and suggestions of the authors. One paper examines a much neglected experimental side of the study of fluorides, their influence on the strength of the altered bone. The best documented beneficial effects of fluorides are found in caries prophylaxis. The paper reviewing this field demonstrates that there is only a partial parallel with bone.

The possible toxic effect of fluorides are likely to cause public concern as observed in the past in relation to caries prophylaxis and fluorosis. It was therefore appropriate to include a chapter about the chronic toxicity of

anorganic fluorides In the second section, four papers deal with different aspects of fluorosis. High dose ingested during many years may lead to fluorosis, and in some cases to its crippling form. The doses necessary to induce these alterations are extremely high and the ingestion period must be long, both in excess of the therapeutically used values.

The third section of this volume deals with fluoride in the treatment of osteoporosis. The evaluation of the effect of therapeutic agents in osteoporotic patients is a difficult task requiring long-term follow-ups and elaborate studies. Three new series of patients adequately studied by bone biopsies and partly with metabolic studies are reported. In contrast to reports on other drugs, there seems to be a beneficial effect of fluoride. But several problems arise such as treatment-schedules, dosages and the possible need for additional therapy.

The last paper critically reviews some of the facts presented in this volume, emphasizing the problems which should be solved in controlled studies before fluorides can be recommended for use. The papers included in this volume are not aimed at giving a definitive answer whether fluorides will ever be useful in treating osteoporosis. But perhaps they show where we stand at this moment and they may be helpful as an up-to-date reference source providing ideas for future research in this promising field.

T. L. VISCHER

AUTHORS' LIST

Prof. G. Ahrens, Universitätsklinik und Poliklinik für Zahn-, Mund- und Kieferkrankheiten, Abteilung C II, Martinistraße 52, D-2 Hamburg 20, Germany

Dr. H. Alami, Institut de morphologie, Université de Genève, CH-1200 Genève, Switzerland

Dr. S. Bano, Institut de morphologie, Université de Genève, CH-1200 Genève, Switzerland

Prof. C.A. Baud, Institut de morphologie, Université de Genève, CH-1200 Genève, Switzerland

Dr. D. L. Baylink, Veteran's Administration Hospital, 4435 Beacon Avenue So, Seattle, Washington, 98108, USA

Dr. C. Bernheim, Policlinique universitaire de médecine, CH-1200 Genève, Switzerland

Prof. G. Cimasoni, Institut de médecine dentaire, 30, rue Lombard, CH-1200 Genève, Switzerland

Prof. B. Courvoisier, Service de médecine, Hôpital de La Chaux-de-Fonds, CH-2300 La Chaux-de-Fonds, Switzerland

Prof. J. P. Dustin, Organisation mondiale de la santé, CH-1200 Genève, Switzerland

Dr. L. Eckermeier, I. Medizinische Universitätsklinik, Universitätskrankenhaus Eppendorf, Martinistraße 52, D-2 Hamburg 20, Germany

Dr. E. Flist, Veteran's Administration Hospital, 4435 Beacon Avenue So, Seattle, Washington, 98108, USA

PD Dr. C. Guerdjikoff, Caisse nationale suisse d'assurance en cas d'accidents, CH-1200 Genève, Switzerland

Dr. C. Jacot, Hôpital de La Chaux-de-Fonds, CH-2300 La Chaux-de-Fonds, Switzerland

Prof. G.N. Jenkins, University of New Castle upon Tyne, Dental School, Upper Claremont Street, New Castle upon Tyne, England

Prof. S.S. Jolly, Rajendra Hospital, Patiala, India

Dr. L. Kruse, I. Medizinische Universitätsklinik, Universitätskrankenhaus Eppendorf, Martinistraße 52, D-2 Hamburg 20, Germany

Prof. F. KUHLENCORDT, I. Medizinische Universitätsklinik, Universitätskrankenhaus Eppendorf, Martinistraße 52, D-2 Hamburg 20, Germany

PD Dr. R. LAGIER, Institut de pathologie, 40, bd de la Cluse, CH-1200 Genève, Switzerland

Prof. W. LEEMANN, Veterinär-Medizinische Klinik der Universität Zürich, Winterthurer Straße 260, CH-8057 Zürich, Switzerland

Dr. C. LOZANO-TONKIN, I. Medizinische Universitätsklinik, Universitätskrankenhaus Eppendorf, Martinistraße 52, D-2 Hamburg 20, Germany

Prof. E MARTIN, Policlinique universitaire de médecine, 24, rue Micheli du Crest, CH-1200 Genève, Switzerland

Dr. W.A. MERZ, Anatomisches Institut der Universität, Pestalozzistraße 20, CH-4000 Basel, Switzerland

Dr. M. PAJAROLA, Medizinische Klinik, Kantonsspital, CH-9006 St.Gallen, Switzerland

Prof. G PETERS, Institut de pharmacologie de l'université, Bugnon 21, CH-1000 Lausanne, Switzerland

Dr F W. REUTTER, Medizinische Klinik, Kantonsspital, CH-9006 St.Gallen, Switzerland

Prof. C RICH, Veterans Administration Hospital, 4435 Beacon Avenue So, Seattle, Washington, 98108, USA

Prof. R K SCHENK, Anatomisches Institut der Universität, Pestalozzistraße 20, CH-4000 Basel, Switzerland

PD Dr R SIEBENMANN, Pathologisches Institut, Kantonsspital, CH-9006 St.Gallen, Switzerland

Dr MARTHA STAUFFER, Veterans Administration Hospital, 4435 Beacon Avenue So, Seattle, Washington, 98108, USA

Dr M THIÉBAUD, Clinique universitaire de chirurgie, Hôpital cantonale, CH-1200 Genève, Switzerland

Dr T L VISCHER, Medizinische Universitätsklinik, Burgerspital, CH-4000 Basel, Switzerland

Prof. P WETTSTEIN, Institut universitaire de radiologie, Hôpital cantonal, CH-1200 Genève, Switzerland

Dr. J WERGEDAHL, Veterans Administration Hospital, 4435 Beacon Avenue So, Seattle, Washington, 98108, USA

Dr R ZENDER, Hôpital de La Chaux-de-Fonds, CH-2300 La Chaux-de-Fonds, Switzerland

Mode of Action of Fluoride

Fluoride and enzymes*

G. CIMASONI

The changes which occur in chronic fluorosis, such as mottling of teeth and skeletal effects, are probably secondary to cellular disfunction, caused by toxic levels of fluoride in the vicinity of the cells. This interfearence of cell function may be related to inhibitory effects of fluoride on specific enzyme systems. With this rather optimistic premise in mind, we will try to discuss the problem of the inhibition of various enzymes by fluoride and see what kind of practical conclusions one can draw from this type of research.

SENSITIVITY OF ENZYMES TO FLUORIDE

When one studies the inhibition of an enzyme, one can first investigate the problem of sensitivity, in term of doses of fluoride needed for liminal and for 50% inhibition. The activity of the enzyme is determined in the presence of increasing concentrations of the halide ion, and the results are usually expressed in percentages of remaining activity, as a function of the logarithm of the fluoride concentration.

Two examples of results obtained in such investigations are shown in Fig. 1 and Fig. 2.

Fig. 1 shows the percentages of remaining activity of a preparation of erythrocyte cholinesterase, in the presence of increasing concentrations (log) of fluoride.

Fig. 2 shows the activities of a preparation of bean urease, in the presence of various concentrations of fluoride. In both cases a straight line was established on the semilogarithmic plot, and the pI 50 values could be determined.

It has been usually shown that the concentration of halide ions needed for the liminal inhibition of enzymes in vitro is higher than that which has been

* The investigations in the author's laboratory were supported by the Swiss national fund for scientific research (grants n. 2478 and 4908.3), and by the Swiss odontological society. The help of Dr. D.A.B. YOUNG and Prof. E. STEIN is gratefully acknowledged.

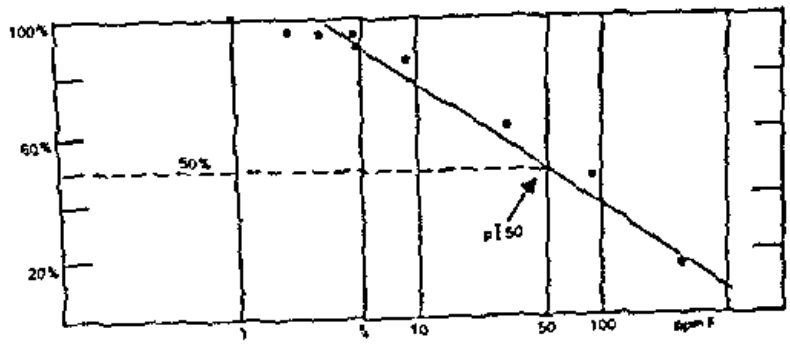


Fig. 1. Activities of a preparation of bovine red blood cell acetylcholinesterase in the presence of increasing concentration of fluoride. The liminal concentration of fluoride, needed for enzyme inhibition, is between 1 and 5 ppm. The pI_{50} value of this enzyme preparation is 50 ppm.

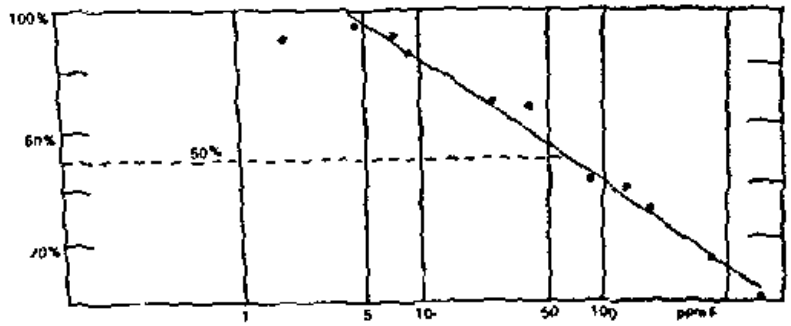


Fig. 2. Activities of a preparation of bean urease in the presence of increasing concentration of fluoride About 5 ppm F^- are needed to start inhibition of this enzyme in vitro, and the pI_{50} value is about 50 ppm.

reported to occur in the blood of people drinking fluoridated water (2, 29, 32) Precise data on this point can be found in the literature concerning enolase (31), acid phosphatase (31), cholinesterases (9), carboxylase (15), lipase (15) There are also enzymes whose activity is stimulated in the presence of low concentrations of fluoride (4).

RAPIDITY OF FLUORIDE INHIBITORY ACTION

It is usually observed that as soon as fluoride is introduced in the medium of the enzymatic assay, inhibition of the fluoride sensitive enzyme will immediately take place.

Fig. 3 and 4 give two examples of this phenomenon, the first with acetylcholinesterase, the second recently observed with enolase in our laboratory.

In both cases, the method used for the determination of enzymatic activity was a photometric kinetic assay, whereby the increasing concentration of the products of reaction are measured with time. One can notice that the

Fluoride and enzymes*

G. CIMASONI

The changes which occur in chronic fluorosis, such as mottling of teeth and skeletal effects, are probably secondary to cellular disfunction, caused by toxic levels of fluoride in the vicinity of the cells. This interfearence of cell function may be related to inhibitory effects of fluoride on specific enzyme systems. With this rather optimistic premise in mind, we will try to discuss the problem of the inhibition of various enzymes by fluoride and see what kind of practical conclusions one can draw from this type of research.

SENSITIVITY OF ENZYMES TO FLUORIDE

When one studies the inhibition of an enzyme, one can first investigate the problem of sensitivity, in term of doses of fluoride needed for liminal and for 50% inhibition. The activity of the enzyme is determined in the presence of increasing concentrations of the halide ion, and the results are usually expressed in percentages of remaining activity, as a function of the logarithm of the fluoride concentration.

Two examples of results obtained in such investigations are shown in Fig. 1 and Fig. 2.

Fig. 1 shows the percentages of remaining activity of a preparation of erythrocyte cholinesterase, in the presence of increasing concentrations (log) of fluoride.

Fig. 2 shows the activities of a preparation of bean urease, in the presence of various concentrations of fluoride. In both cases a straight line was established on the semilogarithmic plot, and the pI 50 values could be determined.

It has been usually shown that the concentration of halide ions needed for the liminal inhibition of enzymes in vitro is higher than that which has been

* The investigations in the author's laboratory were supported by the Swiss national fund for scientific research (grants n. 2478 and 4908.3), and by the Swiss odontological society. The help of Dr. D.A.B. YOUNG and Prof. E. STEIN is gratefully acknowledged.

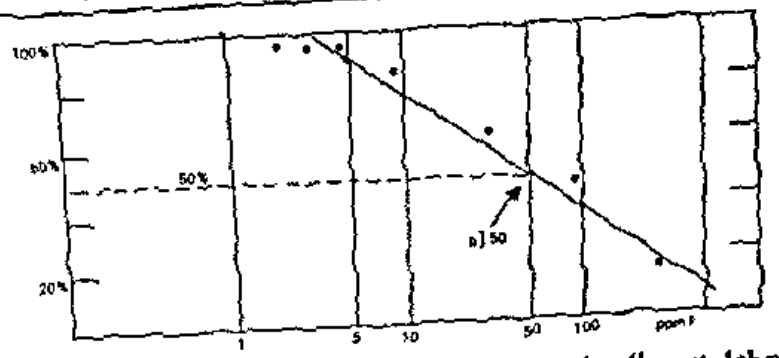


Fig. 1. Activities of a preparation of bovine red blood cell acetylcholinesterase in the presence of increasing concentration of fluoride. The luminal concentration of fluoride, needed for enzyme inhibition, is between 1 and 5 ppm The pI_{50} value of this enzyme preparation is 50 ppm

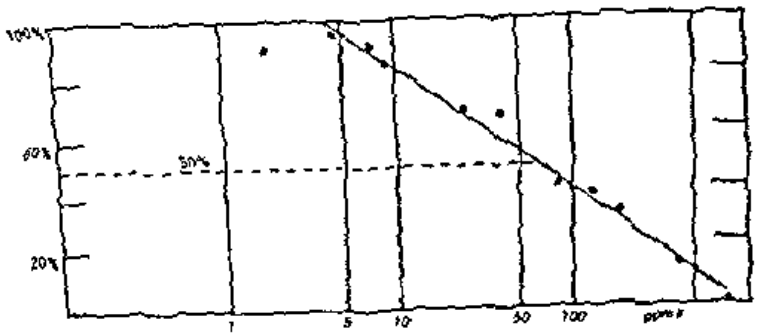


Fig 2 Activities of a preparation of bean urease in the presence of increasing concentration of fluoride About 5 ppm F^- are needed to start inhibition of this enzyme in vitro, and the pI_{50} value is about 50 ppm

reported to occur in the blood of people drinking fluoridated water (2, 29, 32) Precise data on this point can be found in the literature concerning enolase (31), acid phosphatase (31), cholinesterases (9), carboxylase (15), lipase (15). There are also enzymes whose activity is stimulated in the presence of low concentrations of fluoride (4)

RAPIDITY OF FLUORIDE INHIBITORY ACTION

It is usually observed that as soon as fluoride is introduced in the medium of the enzymatic assay, inhibition of the fluoride sensitive enzyme will immediately take place.

Fig 3 and 4 give two examples of this phenomenon, the first with acetylcholinesterase, the second recently observed with enolase in our laboratory.

In both cases, the method used for the determination of enzymatic activity was a photometric kinetic assay, whereby the increasing concentration of the products of reaction are measured with time One can notice that the

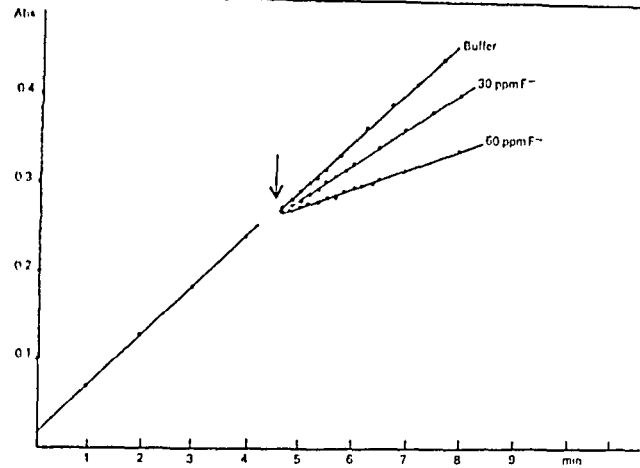


Fig. 3. Rapidity of the inhibitory action of fluoride upon acetylcholinesterase. During the photometric assay, the introduction of fluoride in the reaction mixture is followed by an immediate decrease of the slope measuring the activity of the enzyme. Abscissae: time (minutes). Ordinate: absorbancy at 412 mμ.

presence of fluoride causes an immediate change in the slope measuring the enzyme activity.

REVERSIBILITY

Although the literature is not abundant on this particular point, a third important character of the inhibition of enzymes by fluoride seems to be its reversibility: if one dialyses an inhibited enzyme, if one passes it through a column of Sephadex or Retard-ion resin, if one simply dilutes the inhibited enzyme in a medium without fluoride, one will observe a reversal of the inhibition.

Fig. 5 presents the results of a study of reversibility of the inhibition of acetylcholinesterase by fluoride, obtained by dialysis (9).

Here again a practical implication can be pointed out, pertaining to observations of enzyme inhibition in vivo. In experimental studies, enzymes are usually measured in blood or tissue homogenates after dilution in suitable buffers. If an animal has been exposed to toxic levels of fluoride, it would be misleading to measure its enzymes by classical methods, since dilution could lead to the at least partial reversal of the inhibition under study, which could actually be present in vivo.

For such studies one has therefore to use methods of enzymatic assays in which the enzyme is kept in the same biological medium, in which it is found in vivo.

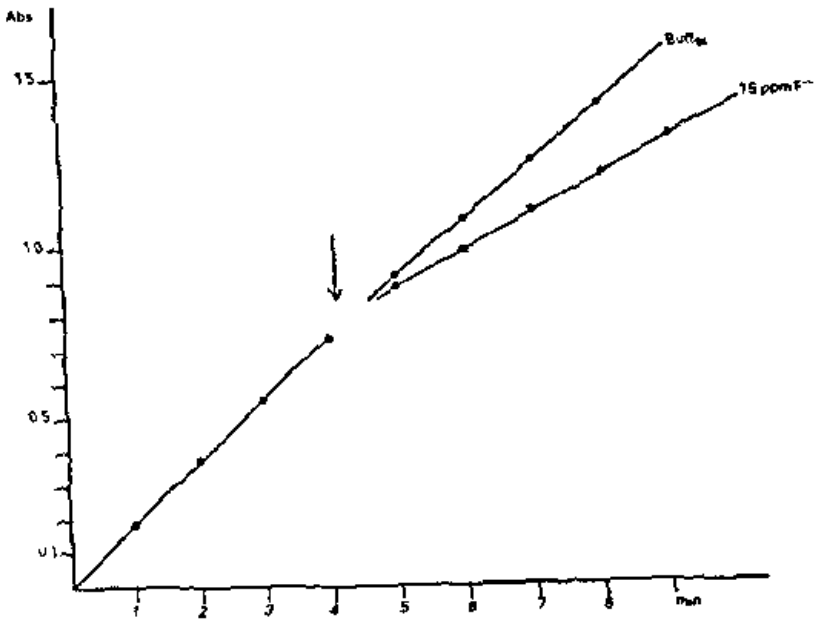


Fig 4 Rapidity of the inhibitory action of fluoride upon enolase. Abscissea: time (minutes) Ordinate absorbancy at 240 mμ

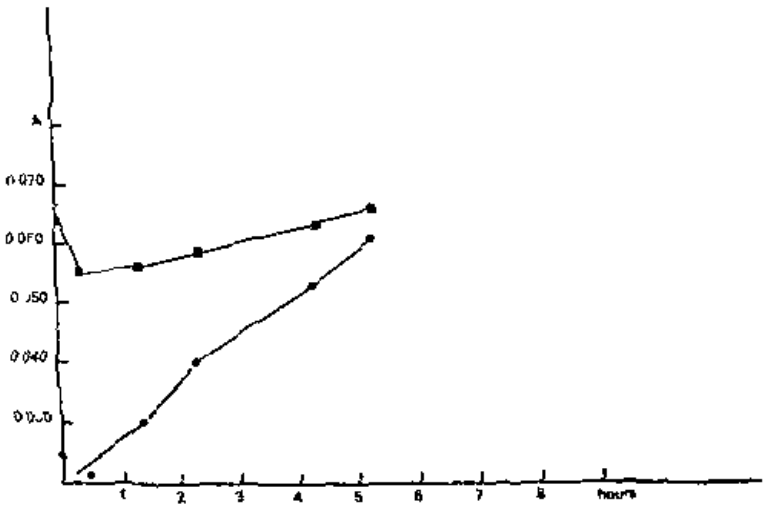


Fig 5 Reversibility of fluoride inhibition of acetylcholinesterase by dialysis
- Activity of a preparation of enzyme in the presence of 90 ppm of fluoride at time *zero, and dialysed against a suitable phosphate buffer.*
- Activity of a control preparation, in the absence of fluoride, similarly dialyzed against buffer

Abscisse time of dialysis (hours).
Ordinate enzyme activities (slope or Δ A)

MECHANISM

As for the mechanism of enzyme inhibition by fluoride, this problem has been for many years dominated by the *model* first proposed by WARBURG and CHRISTIAN in 1942 (34, 5) These authors observed, with crystalline

enolase from yeast, that fluoride inhibition resulted from the formation of a magnesium-fluorophosphate complex. The mechanism was confirmed by MILLER in 1958 (22) and, in view of the importance of the enolase reaction as an energy producing step in glycolysis in a multitude of vegetal and animal organisms, it was postulated as a basic mode of action of fluoride by toxicologists, bacteriologists and dental investigators (15, 33).

Since a variety of phosphate ester splitting enzymes also require Mg^{++} as activator, the blocking of those enzymes by fluoride was always investigated under the hypothesis of the magnesium-fluorophosphate complex. This has been in particular the case of phosphoglucomutase, the enzyme catalysing a reaction (glucose 6-phosphate → glucose 1-phosphate), which is important not only in mammalian carbohydrate metabolism (23), but also in vegetal growth, as the starting point of polysaccharide synthesis for the construction of new cell walls (25).

In 1964, PETERS, SHORTHOUSE and MURRAY (26) have cast some doubts on the demonstration of WARBURG and CHRISTIAN, by showing that enolase, from either muscle or plants, was not inhibited by fluorophosphate, a divalent ion ($F.PO_3^{=}$) capable however of forming a complex with magnesium. In a medium containing 30 m M of Mg^{++}, these authors have shown that enolase could be inhibited up to almost 100% by a concentration of 5 mM of F^- (95 ppm) and in the presence of 5 mM of phosphate.

When fluoride and phosphate were however replaced by 5 or even 10 mM of fluorophosphate, no inhibition of the enzyme occurred.

The demonstration of this group of English biochemists, that the formation of a magnesium-fluorophosphate complex could also not be the real mechanism of enolase inhibition by fluoride, was corroborated by the findings that other enzymes, which do not need any metal ion cofactors, can strongly be inhibited by the halide. This is for instance the case of 5-adenylic acid-deaminase from muscle, as shown by NIKIFORUK and COLOWIK (24) and of cholinesterase and urease, both of which have been studied in detail in our laboratory (9, 13).

Using a preparation of acetylcholinesterase that we had purified by column chromatography on DEAE cellulose, we could show that this enzyme is inhibited by fluoride in the absence of magnesium and phosphate (9). Urease, another enzyme without metal ion cofactors, is also inhibited by fluoride, with a mechanism which seems to be quite similar to that of the cholinesterase. In this investigation, the enzyme preparation was purified through Sephadex G 25 and thus completely deionized: again, the enzyme could be inhibited by fluoride in the absence of phosphate and magnesium (13).

Studies on the kinetics of enzyme inhibition by fluoride are not abundant.

In 1952, SLATER and BONNER have shown that the inhibition of succinic dehydrogenase by fluoride is competitive with respect to the substrate, when phosphate ions are also present (30). Using the classical plot of LINEWEAVER and BURK (21), these authors have shown that the lines representing enzyme activities, in the absence or in the presence of fluoride, are converging on the ordinate axis In the absence of phosphate, very little inhibition could be observed by the halide Phosphate alone could also slightly inhibit the enzyme, and a competition between fluoride and phosphate for the enzyme active site could be established. Interestingly enough, adding magnesium or manganese to the reaction mixture had no effect on the inhibition by fluoride and phosphate

An inhibition also of the competitive type was shown in 1955 by REINER, TSUBOI and HUDSON, who studied the interaction of fluoride with prostatic acid phosphatase.

In this case, no phosphate was added to the reaction medium, but one could argue that some inorganic phosphate was present as the product of the reaction Oxalate and citrate could be shown to reduce to a great extent the inhibition of acid phosphatase by fluoride, which suggested to the authors either a combination of those ions with fluoride, or a competition with the halide ion, for a positively charged group on the active site of the enzyme (27)

This second hypothesis confirms the findings of SLATER and BONNER concerning the interaction of fluoride and phosphate on a common active site of succinic dehydrogenase

As already mentioned, cholinesterase and urease are inhibited by fluoride in the absence of phosphate and magnesium.

The points on the lower line represent the reciprocal values of the enzyme activities found in the presence of increasing concentrations of substrate. (Fig. 6)

The points on the upper line correspond to the reciprocal values of enzyme initial velocities, found with the same substrate concentrations, but in the presence of 45 ppm fluoride. The lines are parallel, indicating a rare type of inhibition, the so-called uncompetitive inhibition (35). Parallel lines were also obtained on the so-called DIXON plot, as shown in Fig. 7, thus confirming the above findings.

These results are interpreted as the consequence of the binding of fluoride to the complex of the enzyme with the substrate (35, 17) They confirm a mechanism of inhibition by fluoride which had already been described by

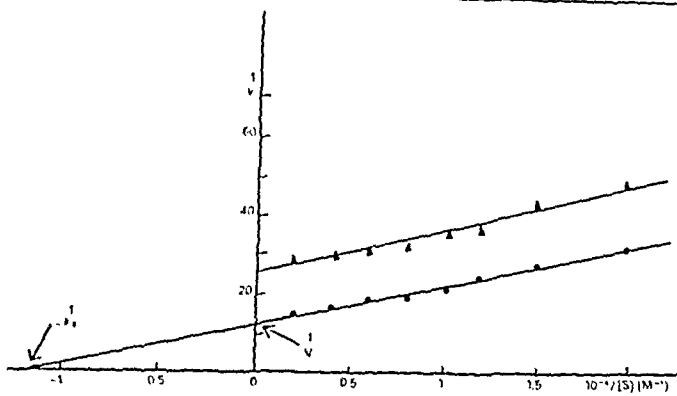


Fig. 6. LINEWEAVER-BURK plots of human red blood cell acetylcholinesterase activity: ● in pure phosphate buffer; ▲ in the presence of 45 ppm fluoride. Eight substrate concentrations were used. $10^3 \times \Delta$ A/min. was used as a measure of v. (Reproduced with the permission of the editors of the Biochemical Journal, reference No. 9.)

CURZON in 1960, concerning the action of fluoride on caeruloplasmin (11). Similar kinetic data have been found in our laboratory on the inhibition of urease by fluoride (13). LEE and WANG have recently studied the kinetics of the inhibition of 5-adenilic acid deaminase by fluoride and confirmed our observations on cholinesterase and urease. In the absence of ATP, which is a potentiator of this deaminase, the inhibition of the enzyme by fluoride was found by these authors to be of the non-competitive type (lines converging on the abscissae axis on the LINEWEAVER-BURK plot). In the presence of ATP, the inhibition was uncompetitive, with parallel lines on the aforementioned plot (20).

It is known that kinetic data can only give a partial and indirect picture of the actual mechanism of inhibition. Furthermore, no data are available, to our knowledge, concerning the kinetics of inhibition of enolase by fluoride. In spite of these limitations, a tentative explanation and summary of our present knowledge on the mechanism of inhibition of enzymes by fluoride is given in Fig. 8.

In the upper part of Fig. 8 one can see the schematic formation of an enzyme-substrate (ES) complex, such as it is thought to be formed, as passing intermediate step, in any enzyme catalyzed reaction. The ES complex is formed, for some enzymes, only in the presence of a metal ion cofactor. Its formation is followed by the appearance of the products of the reaction, while the enzyme remains unchanged.

In the presence of fluoride, if we exclude the formation of the magnesium fluorophosphate complex, three possibilities seem to occur, according to the

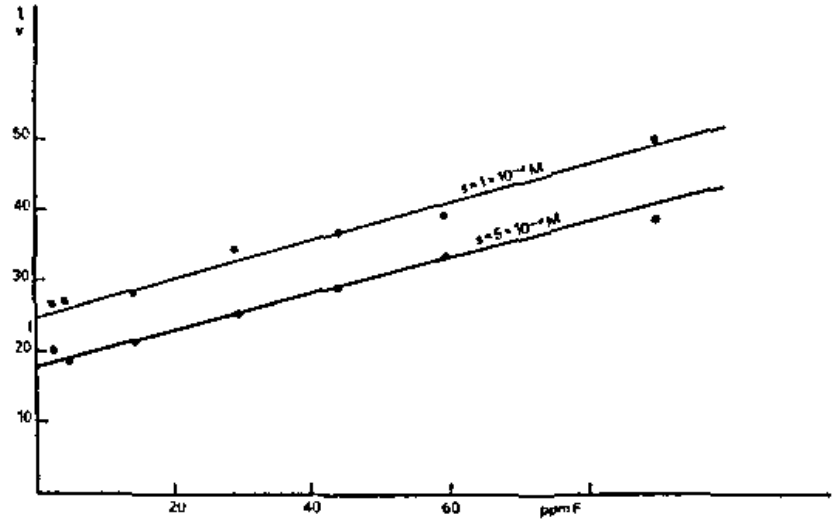


Fig 7. Dixon ($\frac{1}{v}$-F^-) plot of human red blood cell acetylcholinesterase activity in the presence of two substrate concentrations.

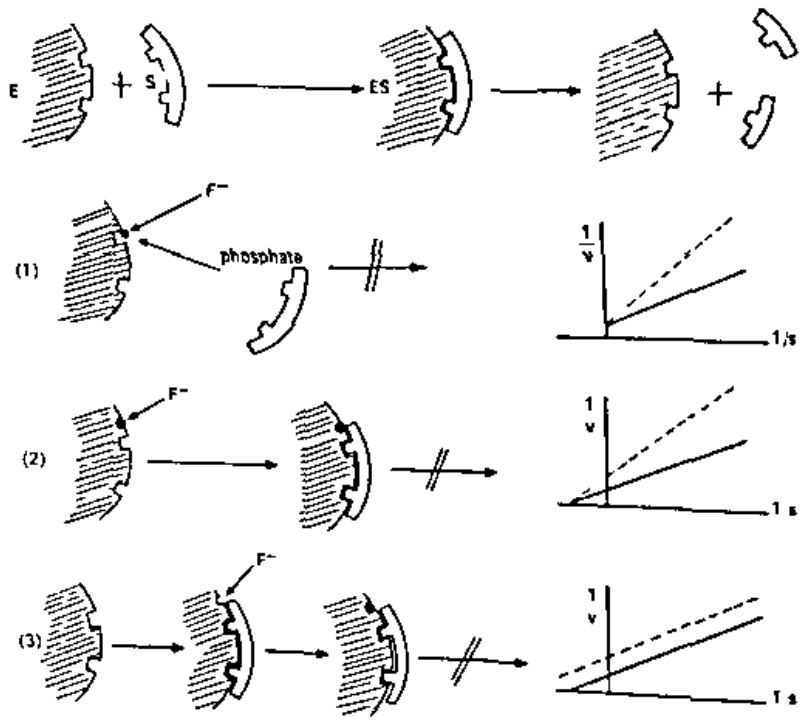


Fig. 8 Tentative, schematic representation of the three possible mecanisms by which enzymes can be inhibited by fluoride. For explanation see the text

what is known

competes with an anion, possibly phosphate, for a group on the active site of the enzyme. The inhibition is therefore competitive. Example, the inhibition of succinic dehydrogenase by fluoride (30)

(2) The halide binds directly to the enzyme molecule, not at the active site, and therefore does not hinder the formation of an ES complex This

ES complex will however not be dissociated, and the reaction is therefore inhibited (non-competitive inhibition). Example: the inhibition of 5-adenylic acid deaminase in the absence of ATP (20).

(3) The halide will bind to the enzyme, only after this has undergone the conformational changes imposed by the substrate. This ES complex, bearing the fluoride, will also remain undissociated, and the reaction is therefore inhibited (uncompetitive inhibition). Examples: the inhibition of cholinesterases, of urease, of 5-adenylic acid deaminase in the presence of ATP, of caeruloplasmin (9, 13, 20, 11).

Studies are now under way, by using techniques of gel filtration, which should allow the more direct demonstration of some of the mechanisms just illustrated (10).

FLUORIDE AND THE CELL

The action of fluoride on enzymes may be the basic mechanism by which one can explain many of the pharmacological effects of the halide. However, no major conclusions can be drawn from this area of research, until the action of fluoride will be thouroughly determined on metabolic systems as they actually occur in the cell.

At least four points should be mentioned in this part of the discussion:
(1) The passage of fluoride in the intracellular fluid.
(2) The effect of fluoride upon the permeability of cellular membranes.
(3) The effects of fluoride upon ion transport across cellular membranes.
(4) The effects of fluoride upon cell growth.

–As for the first point, passage of fluoride in the intracellular space, CARLSON, SINGER and ARMSTRONG have shown that this varies with the nature of tissue (6). After administration of radioactive fluoride to rats, the authors have determined in various soft tissues and in plasma, the radiofluoride concentrations, expressed in counts per microequivalents of chloride. They have then determined the ratios of radiofluoride concentrations, so expressed, in various organs to that of plasma. Since chloride is essentially an extracellular ion, a ratio greater than one indicates a penetration of fluoride into the intracellular compartment. Muscle, liver and tendon showed values above 2, but brain tissue had the lowest ratio (0.4), indicating a barrier to the passage of fluoride into the intracellular space. Data concerning hard tissues were not available in this study, but the well known sensitivity of bone cells to fluoride could profitably be investigated in terms

of intracellular passage. In studying the inhibition of yeast growth by fluoride, ROTHMAN and CABIB (28) have reported that the permeability of these cells to fluoride is practically suppressed when the pH passes from 5.2 to 6.4. This could explain the well known greater sensitivity of salivary microorganisms to fluoride at pH 5 than at neutrality (18).

– Fluoride probably affects the permeability of cellular membranes. Concerning this second point, WILBRANDT showed in 1940 that 20–40 mM of fluoride causes human erythrocytes to shrink and become resistant to osmotic haemolysis, due to rapid loss of potassium from the cell (36). A rapid increase in efflux of potassium was also shown, under the influence of fluoride, by DANIEL (12), who has used rat uterin horns, in vitro. Finally, a leakage of nucleotides from the cell has also been postulated by CARLSON and SUTTIE as an explanation for the decrease in ATP concentration that these authors have reported in HeLa cells exposed to 30 ppm F^- (8).

– Changes in permeability due to fluoride could also be the consequence of a modified ion transport mecanism across cellular membranes. An inhibition of sodium extrusion from erythrocytes has been reported by KIRSCHNER in the presence of $4\,6 \times 10^{-3}$ M fluoride (87 ppm) (16). It is known that this effect is due to the inhibition of the Na^+ and K^+ dependent ATPase, which is considered to participate in the active ion transport (37). An inhibition of this enzyme was noticed already at 0.3 mM fluoride (5.7 ppm).

– Finally, recent studies using cultures of cells have allowed direct observations on the action of fluoride upon cellular growth and metabolism

cells (3), and by ALBRIGHT, who studied fluoride action upon growth of leukemic lymphoblasts (1). Experiments using ^{14}C-labelled glucose in cultures of HeLa cells have shown that [illegible] as well as CO_2 production are not [illegible] of fluoride (30 ppm). In spite of this lack of action by fluoride upon glycolysis and KREBS cycle, the cells growing in such medium showed a decrease in the concentration of ATP, which could, as already pointed out, be due to a leakage of nucleotides from the cells (8).

CONCLUDING REMARKS

The data presented in our last chapter should be a valid demonstration that the basic action of fluoride upon cellular metabolism cannot be explained only in terms of effects upon various enzyme systems. This consideration is confirmed by the investigators who have studied the influence of the halide ions upon the metabolism of salivary bacteria, as a possible explanation of its cariostatic action (19).

It is however not excluded that, with the progress which is being made on the understanding of the nature and location of intracellular metabolic phenomena, the peculiar property of fluoride as an inhibitor of enzymes could one day lead to a rewarding result.

REFERENCES

1 ALBRIGHT, J.A.: Inhibitory levels of fluoride on mammalian cells. Nature *203*, 976 (1964).
2 ARMSTRONG, W.D., SINGER, L., EINSINCK, J., and RICH, C.: Plasma fluoride concentrations of patients treated with sodium fluoride. J. Clin. Invest. *43*, 555 (1964).
3 ARMSTRONG, W.D.: Sodium fluoride and cell growth. Brit. Med. J. *1*, 1435 (1965).
4 ARRIGONI, O., and MARRÈ, E.: Effect of adenosine phosphates on the direct oxidation of glucose-6-phosphate by plant extracts. Giorn. Biochim. *4*, 1 (1955).
5 BOREI, H.: Inhibition of cellular oxidation by fluoride. Arkiv. Kemi. Mineral. Geol. *20* A (8), 215 (1945).
6 CARLSON, C.H., SINGER, L., and ARMSTRONG, W.D.: Radiofluoride distribution in tissues of normal and nephrectomized rats. Proc. Soc. Exp. Biol. Med. *103*, 418 (1960).
7 CARLSON, J.R., and SUTTIE, J.W.: Effects of sodium fluoride on HeLa cells. I. Growth, sensitivity and adaptation. Exp. Cell. Res. *45*, 415 (1967).
8 CARLSON, J.R., and SUTTIE, J.W.: Effects of sodium fluoride on HeLa cells. II. Metabolic alterations associated with growth inhibition. Exp. Cell. Res. *45*, 423 (1967).
9 CIMASONI, G.: Inhibition of Cholinesterases by fluoride in vitro. Biochem. J. *99*, 133 (1966).
10 CIMASONI, G., and YOUNG, D.A.B.: The binding of fluoride to albumin as studied by gel filtration. Orca Congress, Basle, July 8–10 (1968).
11 CURZON, G.: The effects of some ions and chelating agents on the oxidase activity of caeruloplasmin. Biochem. J. *77*, 66 (1960).
12 DANIEL, E.E.: Potassium movements in rat uterus, studied in vitro. II. Effects of metabolic inhibitors, ouabain and altered potassium concentration. Canad. J. Biochem. Physiol. *41*, 2085 (1963).
13 DESCOEUDRES, A., and CIMASONI, G.: The inhibition of urease by fluoride in vitro. I.A.D.R. Continental european division. Fifth annual meeting. Roma, Oct. 5, 6 (1968).

14 HEWITT, E J., and NICHOLAS, D J D.: *Cations and anions: inhibitors and* interactions in metabolism and in enzyme activity. In: Metabolic Inhibitors, vol II, ch 29, p. 311. Ed by: HOCHSTER, R.M , and QUASTEL, J H. Academic Press (1963)

15 HODGE, H.C , and SMITH, F.A , in: Fluorine Chemistry Ed. by SIMONS, J.H. Academic Press, N.Y. and London, p 176 (1965).

16 KIRSCHNER, L B · Fluoride inhibition of sodium extrusion from swine erythrocytes and its metabolic correlates Archs Biochem. Biophys *106*, 57 (1964).

17 KRUPKA, R M . Fluoride inhibition of acetylcholinesterase. Mol. Pharmacol. *2*, 558 (1966).

18 JENKINS, G.N. The effect of pH on the fluoride inhibition of salivary acid production. Arch. Oral Biol *1*, 33 (1959)

19 JENKINS, G N , FERGUSON, D.B , and EDGAR, W.M. Fluoride and the metabolism of salivary bacteria. Helv Odont Acta *11*, 2 (1967)

20 LEE, Y.P , and WANG, M.H Studies on the nature of the inhibitory action of inorganic phosphate, fluoride and detergents on 5-adenylic acid deaminase activity and on the activation of adenosine triphosphate. J Biol Chem. *243*, 2260 (1968)

21 LINEWEAVER, H , and BURK, D The determination of enzyme dissociation constants. J. Am. Chem Soc *56*, 658 (1934).

22 MILLER, G W Properties of enolase in extracts from pea seeds Plant Physiol. *33*, 199 (1958).

23 NAIJAR, V A The isolation and properties of phosphoglucomutase J. Biol. Chem *175*, 281 (1948)

24 NIKIFORUK, G., and COLOWICK, S P The purification and properties of 5-

yeast

[illegible] phosphate Arch. J. Biol. 39, 669 (1952)

26 PETERS, R A , SHORTHOUSE, M , and MURRAY, L R Enolase and fluorophosphate Nature *202*, 1331 (1964)

27 REINER, J M , TSUBOI, K.K , and HUDSON, P B Acid phosphatase IV Fluoride inhibition of prostatic acid phosphatase Arch Biochem Biophys *56*, 165 (1955)

fluoride and its
(1966).

29 Dent Res *39*,

30 SLATER, E C , and BONNER, W D. The effect of fluoride on the succinic oxidase system Biochem J *52*, 185 (1952)

31 SMITH, Q T , ARMSTRONG, W D., and SINGER, L.. Inhibition of human salivary and prostatic acid phosphatase and yeast enolase by low fluoride concentrations. Proc Soc Exp Biol Med *102*, 170 (1959).

32 TAVES, D R Normal human serum fluoride concentrations Nature *211*, 192 (1966)

33 THOMAS, M D., and ALTHER, E W. · Enzyme inhibition by fluoride. In: Handbuch der experimentellen Pharmakologie, vol XX, Pharmacology of fluoride, ch 5, p 281 Springer-Verlag (1966)

34 WARBURG, O , and CHRISTIAN, W. Isolierung und Kristallisation des Gärungsferments Enolase. Biochem Zschr 310, 384 (1942).

35 Webb, J.L.: Enzyme and metabolic inhibitors. Vol.1. Academic Press (1963).
36 Wildbrandt, W.: Die Abhängigkeit der Ionenpermeabilität der Erythrozyten vom glycolytischen Stoffwechsel. Pflüger Arch. Ges. Physiol. *243*, 519 (1940).
37 Yoshida, H., Nagai, K., Kamei, M., and Nakagawa, Y.: Irreversible inactivation of (Na^+–K^+)-dependent ATPase and K^+-dependent phosphatase by fluoride. B.B.A. *150*, 162 (1968).

Fluoride and bone mineral substance

C. A. Baud and S Bang

Despite great interest in the prevention of dental caries using fluoride, and the recent suggestions that fluoride may be of therapeutic value in metabolic bone disease, little information is available on the metabolism of fluoride (13). The primary difficulties in these studies arise from the lack of data concerning the topographic localization of fluoride in the calcified tissues at the microscopical level, the relationship of fluoride to the hydroxyapatite crystals of the mineral substance, and the possible toxic effects of fluoride on metabolic activity of the bone cells

It is the purpose of this presentation to provide information with respect to these topics The effects of fluoride administration *in vivo* on bone tissue were studied by means of X-ray emission (4, 5) and diffraction (3, 6, 7, 8, 9) analyses, and of electron microscopy*.

1 MICROSCOPIC DISTRIBUTION PATTERN OF FLUORIDE IN BONE TISSUE

In the bones of mice having received 0 03 mg F per day from the age of 2 months to the age of 4.5 months, and sacrificed a few days after the cessation of treatment, the distribution of fluorine as evidenced by electron probe X-ray microanalysis is nonhomogeneous. There is a heavy lay-down in the layers deposited during the treatment, whereas bone tissue existing before the beginning of the fluoride diet shows only a small amount of fluorine (Fig 1). This repartition is quite similar to that of bone seeking isotopes such as calcium, evidenced in autoradiographs as "hot spots" and "diffuse component".

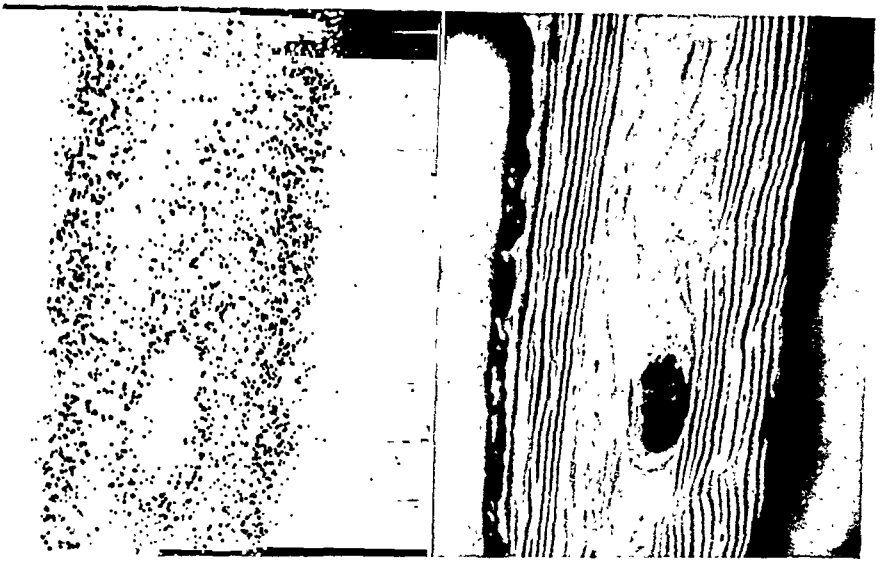


Fig. 1. Fluoride treated mouse. *Left:* The image for the Kα emission wavelength of fluorine from a section of parietal bone, obtained by electron probe X-ray microanalysis. *Right:* Photomicrograph by incident light of the same area. ×200.

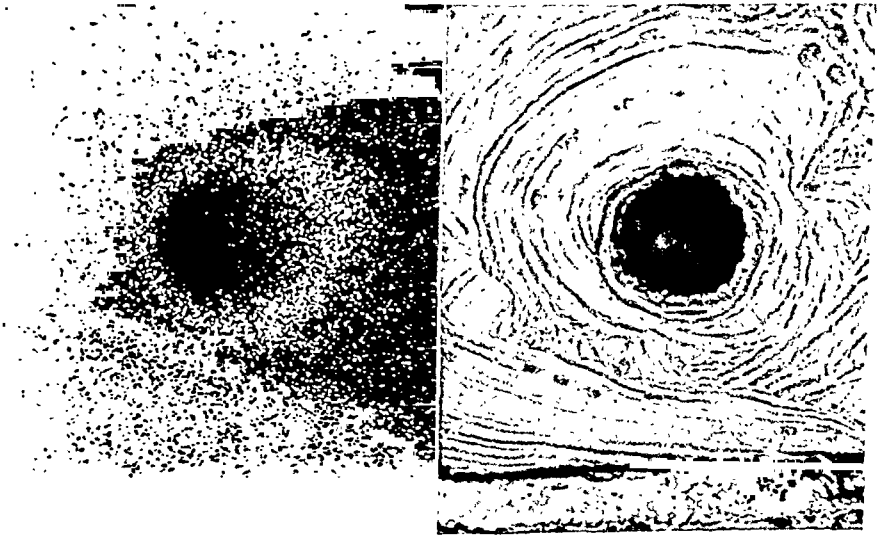


Fig. 2. Fluoride treated osteoporotic patient, six months after the end of treatment. *Left:* The image for the Kα emission wavelength of fluorine from a section of iliac crest, obtained by electron probe X-ray microanalysis. *Right:* Photomicrograph by incident light of the same area. ×200.

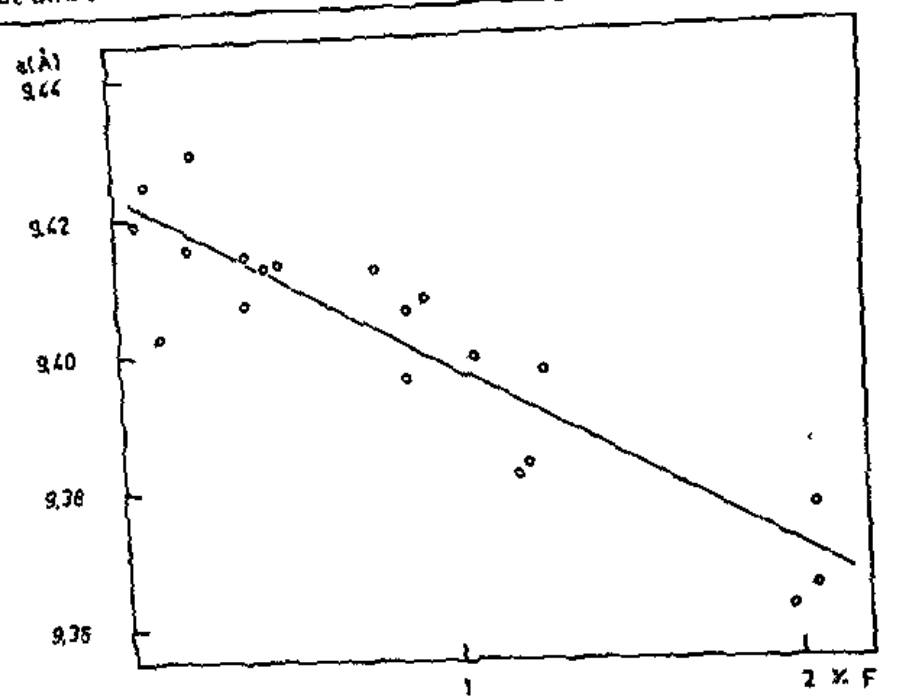


Fig 3 Fluorosed cows. Variation of the parameter *a* of the unit cell of the bone apatite, as a function of its fluorine content, evaluated by high resolution X-ray diffraction analysis

deposited during the treatment having a high level, whereas preexisting bone tissue shows a low amount of F (Fig. 2). The persistence of such a pattern proves that fluorine is firmly bound to the bone substance.

These findings support the autoradiographic observations with radiofluoride, showing that the uptake of fluoride by bone is greater in the sites of active ossification and in the immediate vicinity of blood vessels (2, 13, 16, 24) However, because of the short half life of 18 F (112 min.), most experiments must be entirely completed within about 10 hours, and are unable to demonstrate a long term distribution of F. Moreover, due to the poor resolution of the photographic material, only a macroscopic visualization is obtainable.

2 CRYSTALLOGRAPHIC CHANGES IN THE BONE MINERAL

Samples of compact bone, of which the fluoride content was determined, were studied by high resolution X-ray diffraction (3, 7, 8, 9). The parameter a of the unit cell of the apatite bone crystals was calculated with a precision of $\pm$ 0 001 Å. This analysis shows a shortening of the parameter *a*, in direct linear relation to the increase in amount of F (Fig. 3). The relationship is similar to that observed when the fluoride ions replace hydroxyl ions by

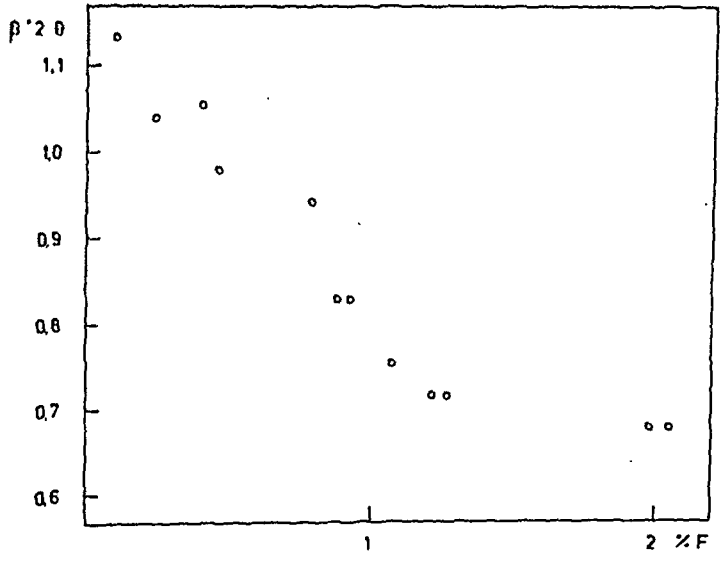


Fig. 4. Fluorosed cows. Relationship between the crystallinity of bone mineral and its fluorine content: β value (the width at half maximum, in degrees 2θ, of the X-ray diffraction peaks) decreases as the crystallinity increases.

isomorphous substitution in the synthetic calcium apatites. The gradual shortening of the parameter *a* shows that *in vivo* fluoride is incorporated inside the crystal lattice; this location explains the stability of the fluoride laid down in the bone.

Another modification of the bone mineral under the influence of fluoride concerns the crystal size. Thirty years ago, REYNOLDS et al. (28) observed that the tibiae of the rats fed on a diet containing 0.1 per cent sodium fluoride gave X-ray diffraction lines slightly thinner than those of control animals, indicating that there is a tendency in the bone for the sodium fluoride to form slightly larger crystals. More recent reports showed that the X-ray diffraction patterns of human (11, 17, 18, 26, 35), bovine (6, 34), rat (21, 22, 23, 30) and mouse (36) bone apatites were sharpened, or better resolved, as the fluorine content increased. This phenomenon was related to an increase in bone apatite crystal size (14, 15, 32). As evidenced by BAUD and MOGHISSI-BUCHS (6), X-ray diffraction analysis of fluorosed bovine bone shows that the crystallinity of the mineral increases markedly as the fluorine content increases from 0 to 1.2 per cent, and very few from 1.2 to 2.0 per cent (Fig. 4).

Fig 5 Fluoride treated mouse Osteocyte showing the dilatation of the rough-surfaced endoplasmic reticulum Electron micrograph, ×8500.

3 ULTRASTRUCTURAL CHANGES IN OSTEOCYTES AND PERILACUNAR AREAS

In the zones of very rapid bone formation, marked alterations in the fine structure of osteocytes of fluoride treated mice are observable in the electron micrographs. The most striking and consistent change is the appearance of large distensions of the rough surfaced endoplasmic reticulum (Fig. 5), sometimes confluent and involving the perinuclear space (Fig 6) The characteristic orientation of the ribosomes attached to the cisternal membranes of the endoplasmic reticulum disappears; the number of free cytoplasmic ribosomes, not grouped in clusters or rosettes, increases. In the perilacunar area, the organic matrix fails to mineralize; the extracellular filamentous material is poorly oriented, and the periodic banding of collagen is not always visible (Fig. 7)

These morphologic alterations characterize at the submicroscopic level the "mottled" bone tissue deposited under the influence of fluoride, easily iden-

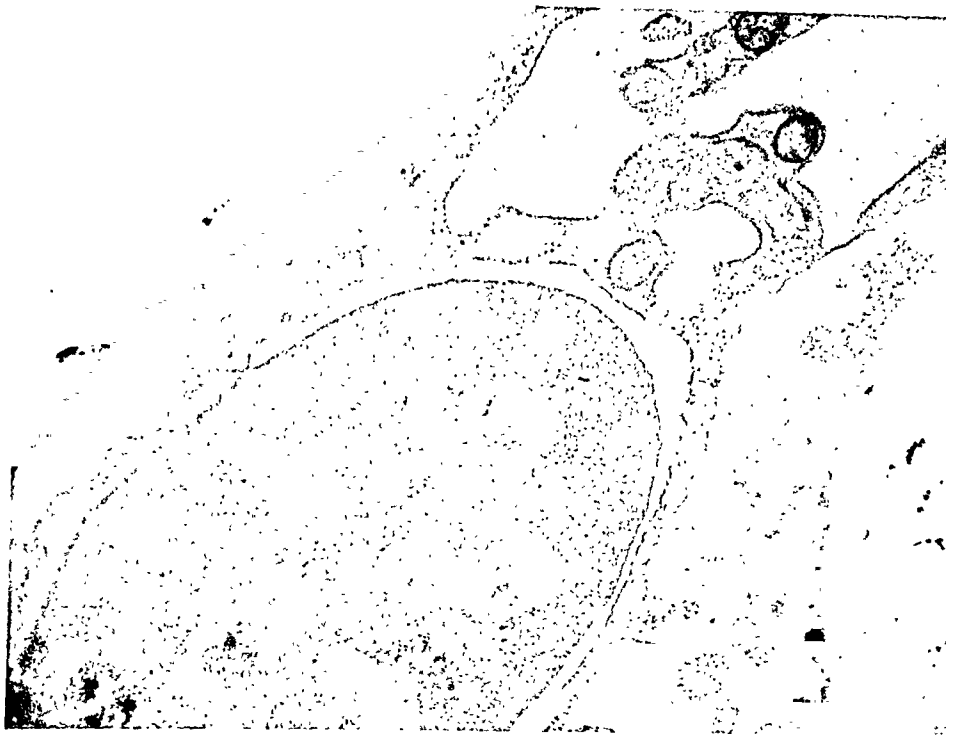


Fig. 6. Fluoride treated mouse. Osteocyte showing large confluent distensions of the rough-surfaced endoplasmic reticulum, involving the perinuclear space. Electron micrograph, ×8500.

tifiable in the microradiographs of fluorosed bone in bovine (19) and humans (1), and after fluorotherapy in osteoporotic patients (33).

The appearances of the endoplasmic reticulum are similar to the degenerative changes already evidenced in ameloblasts from rats which had received repeated doses of fluoride (20). It is also interesting to note that a distortion or dissociation of polyribosomes was previously demonstrated in rabbit reticulocytes incubated with sodium fluoride (12), and in various experimental conditions leading to altered synthesis of the specific proteins produced by the cells (29).

Such changes could well influence not only the quality of the organic perilacunar matrix, but also its mineralization, and explain the presence of the uncalcified or poorly calcified perilacunar "halo" also observed in the electron micrographs (Fig. 8).

Fig. 7. Fluoride treated mouse Uncalcified perilacunar area, showing the poorly oriented extracellular filamentous material. Electron micrograph, ×8500.

Fig 8 Fluoride treated mouse Beside a dead osteocyte, spheritic mineralization in the perilacunar area Electron micrograph, ×8500

SUMMARY

The effects of fluoride administration *in vivo* on bone mineral substance have been studied by means of X-ray emission and diffraction analyses, and of electron microscopy.

Electron probe X-ray microanalysis shows the topographic distribution of F in the bone sections. The layers deposited during the treatment have a high level, whereas bone tissue existing before the beginning of the fluoride diet shows only a small amount of F. This repartition is quite similar to that of bone seeking isotopes evidenced in autoradiographs as "hot spots" and "diffuse component".

High resolution X-ray diffraction shows a shortening of the parameter *a* of the unit cell in the bone apatite crystals, in direct linear relation to the increase in amount of F, suggesting that F is incorporated *in vivo* into the crystal lattice by isomorphous substitution of OH. A relation of bone crystallinity to F concentration is also evidenced.

Marked alterations in the fine structure of osteocytes are produced under the influence of the F diet. Large distensions of the endoplasmic reticulum and distorsions of ribosomal patterns are observed in the electron micrographs. These changes could influence the quality of the organic perilacunar matrix and its mineralization, and explain the presence of the uncalcified or poorly calcified perilacunar "halo" also observed in the electron micrographs.

REFERENCES

1 ADAMS, P. H., and JOWSEY, J.: Sodium fluoride in the treatment of osteoporosis and other bone diseases. Ann. internal Med. *63*, 1151 (1965).

2 APPELGREN, L. E., ERICSSON, Y., and ULLBERG, S.: A comparison of the distribution of radioactive fluorine and calcium by use of double-isotope autoradiography. Acta physiol. Scand. *53*, 339 (1961).

3 BAUD, C. A., et BUCHS, M.: Etude par diffraction des rayons X de la substance minérale de l'os et de l'émail dentaire fluorés in vivo. In: "Tooth enamel", ed. by M. V. STACK and R. W. FEARNHEAD. Bristol, Wright (1965), pp. 32–35.

4 BAUD, C. A., et LOBJOIE, D. P.: L'emploi du radioanalyseur à microsonde électronique pour l'étude de la distribution du fluor dans les coupes d'émail dentaire. Ann. Histochim. *11*, 151 (1966).

5 BAUD, C. A., et LOBJOIE, D. P.: Données de l'analyse élémentaire des tissus calcifiés obtenues avec le radioanalyseur à microsonde électronique. In: "Les tissus calcifiés. 5e Symposium européen, Bordeaux 1967", ed. par G. MILHAUD, M. OWEN, H. J. J. BLACKWOOD. Paris, Sedes (1968), pp. 369–375.

6 Baud, C. A , et Moghissi-Buchs, M.: Données radiocristallographiques sur les changements de cristallinité en rapport avec le taux de fluor incorporé in vivo dans la substance minérale osseuse. C. R. Acad. Sc. Paris *260*, 1793 (1965).
7 Baud, C A , et Slatkine, S · Données radiocristallographiques sur l'incorporation du fluor dans la substance minérale osseuse in vivo. C. R. Acad. Sc. Paris 255, 1801 (1962).
8 Baud, C. A., et Slatkine, S.: Etude par diffraction des rayons X de la fixation in vivo du fluor dans la substance minérale des os. Advances in Fluorine Research and Dental Caries Prevention *2*, 107 (1964).
9 Baud, C. A , and Slatkine, S. Fluoride metabolism and bone mineral substance. In "Calcified tissues Proc. 2nd Europ Symp., Liège 1964", ed. by L. J.
10
11 Bernstein, D. S., and Cohen, P. Use of sodium fluoride in the treatment of osteoporosis. J clin Endocrinol. Metabol *27*, 197 (1967)
12 Conconi, F M , Bank, A , and Marks, P. A. Polyribosomes and control of protein synthesis effects of sodium fluoride and temperature in reticulocytes. J mol Biol *19*, 525 (1966).
13 Dworkin, H J , Moon, N F , Lessard, R. J , and Lafleur, P.· A study of the metabolism of fluorine-18 in dogs and its suitability for bone scanning. J nuclear Med *7*, 510 (1966).
14 Eanes, E D Effects of fluoride on human bone apatite crystals Ann. N Y. Acad Sci *131*, 727 (1965)
15 Eanes, E D , Zipkin, I., Harper, R A , and Posner, A S : Small-angle X-ray diffraction analysis of the effect of fluoride on human bone apatite. Archs oral Biol *10*, 161 (1965)
16 Ericsson, Y , Ullberg, S , and Appelgren, L. E . Autoradiographic localization of radioactive fluorine (F18) in developing teeth and bones. Acta odont. Scand *18*, 253 (1960).
17 Frazier, P D , Zipkin, I , and Mills, L. F X-ray diffraction study of human bone Direct methods of estimating changes in line broadening. Archs oral Biol *12*, 73 (1967)
18 Gron, P , McCann, H G , and Bernstein, D.· Effect of fluoride on human osteoporosis bone mineral A chemical and crystallographic study. J. Bone Joint Surgery *48A*, 892 (1966)
19 Johnson, L C Histogenesis and mechanisms in the development of osteofluorosis In "Fluorine chemistry", ed. by J. H. Simons, vol 4 New York
21 Likins, R. C , Posner, A S., and Pakis, G.: Effect of fluoride on crystal texture and radiocalcium uptake of rat bone. Proc. Soc exper. Biol. Med. *115*, 511 (1964)
22 Menczel, J , Posner, A. S , Schraer, H., Pakis, G., and Likins, R. C.: Comparative fixation of Sr^{89} and Ca^{45} by calcified tissues as related to fluoride induced changes in crystallinity. Proc Soc. exper Biol. Med. *110*, 609 (1962).

23 MLNCZEL, J., SCHRAER, R., PAKIS, G., POSNER, A. S., and LIKINS, R. C.: Effect of a low calcium diet on bone cristallinity and skeletal uptake of Ca^{45} in rats. Proc. Soc. exper. Biol. Med. *112*, 128 (1963).

24 PERKINSON, J. D., WHITNEY, I. B., MONROE, R. A., LOTZ, W. E., and COMAR, C. L.: Metabolism of fluorine 18 in domestic animals. Amer. J. Physiol. *182*, 383 (1955).

25 POSNER, A. S., and EANES, E. D.: Fluoride in bone and teeth. Norelco Reporter *10*, 71 (1963).

26 POSNER, A. S., EANES, E. D., HARPER, R. A., and ZIPKIN, I.: X-ray diffraction analysis of the effect of fluoride on human bone apatite. Archs oral. Biol. *8*, 549 (1963).

27 POSNER, A. S., EANES, E. D., and ZIPKIN, I.: X-ray diffraction analysis of the effect of fluoride on bone. In: "Calcified tissues. Proc. 2nd Europ. Symp., Liège 1964", ed. by L. J. RICHELLE and M. J. DALLEMAGNE. Liège (1965), pp. 79–88.

28 REYNOLDS, L., CORRIGAN, K. E., HAYDEN, H. S., MACY, I. G., and HUNSCHER, H. A.: Diffraction studies of the effect of sodium fluoride and parathormone upon the incisors and tibiae of rats. Amer. J. Roentgenol. *39*, 103 (1938).

29 ROSS, R., and BENDITT, E. P.: Wound healing and collagen formation. IV. Distortion of ribosomal patterns of fibroblasts in scurvy. J. Cell. Biol. *22*, 365 (1964).

30 SCHRAER, H., POSNER, A. S., SCHRAER, R., and ZIPKIN, I.: Effect of fluoride on bone "crystallinity" in the growing rat. Biochim. biophys. Acta *64*, 565 (1962).

31 SLATKINE, S.: Etude biocristallographique de l'incorporation du fluor dans la substance minérale osseuse in vivo. Rev. mens. suisse d'Odonto-stomatologie *72*, 1068 (1962).

32 TANNENBAUM, P. J., and TERMINE, J. D.: Statistical analysis of the effect of fluoride on bone apatite. Ann. N.Y. Acad. Sci. *131*, 743 (1965).

33 THIEBAUD, M., et BAUD, C. A.: Traitement des ostéopathies atrophiques par le NaF. Helv. med. Acta *34*, suppl. *48*, 126 (1969).

34 ZIPKIN, I., EANES, E. D., and SHUPE, J. L.: Effect of prolonged exposure to fluoride on the ash, fluoride, citrate and crystallinity of bovine bone. Amer. J. vet. Res. *25*, 1595 (1964).

35 ZIPKIN, I., POSNER, A. S., and EANES, E. D.: The effect of fluoride on the X-ray-diffraction pattern of the apatite of human bone. Biochim. biophys. Acta *59*, 255 (1962).

36 ZIPKIN, I., SOKOLOFF, L., and FRAZIER, P. D.: A study of the effect of fluoride on bone and osteoarthritis in mice. Israel J. med. Sci. *3*, 719 (1967).

Effects of fluoride on bone formation, mineralization, and resorption in the rat*

D. Baylink, J. Wergedal, M. Stauffer and C. Rich

INTRODUCTION

Osteoporosis, which is characterized by a decrease in bone mass and thus a decrease in bone strength, is a very common metabolic bone disorder. In the U S, more than 4 million people have osteoporosis (17). Because the etiology of osteoporosis is unknown and because there is a substantial amount of morbidity associated with this bone disorder there is considerable interest in empirical forms of therapy.

Although a number of agents have been used to treat osteoporosis, fluoride is the only one which has been shown to increase bone density (24). However, this increase in bone density does not necessarily mean that bone strength is also increased. In fact, patients with osteoporosis treated with high doses of sodium fluoride have been shown by histologic methods to have qualitative abnormalities in bone (3) On the other hand, in our experience there is suggestive evidence that bone pain is reduced in some osteo-

of osteopor - patients using histologic methods (3). We found suggestive evidence for (1) an increased osteoblastic bone formation, (2) an increased osteoclastic resorbing surface, (3) a greater increase in formation than resorption and as a result increased bone mass, and (4) a mineralization defect as evidenced for example by increased osteoid width. Unfortunately we were unable to quantitate the above parameters in human bone biopsies because of a number of technical problems (3). Therefore, in order to obtain

* This study was supported in part by grants from USPHS (AM 9096 and AM 5498).

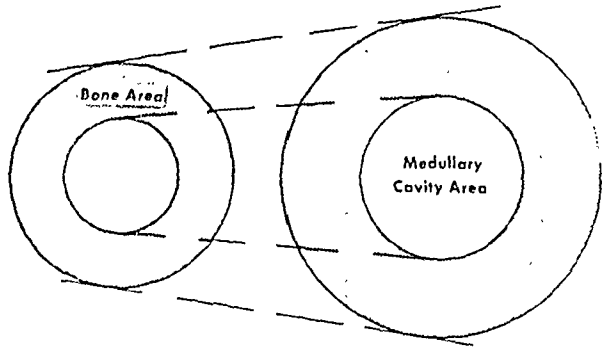


Fig. 1. Schema of the changes which occur in bone area and medullary area during growth in normal rats. Two transverse sections of diaphyseal bone from the tibia are shown; the one on the left is from a weanling rat and the one on the right from an older rat.

quantitative information on the effect of fluoride on bone, we have evaluated these parameters in rats using recently developed quantitative histologic methods for measuring bone formation, mineralization and resorption (5, 6).

The principle of the method used to measure bone formation and resorption is shown schematically in Fig. 1. As illustrated, in young rats during growth there is a progressive increase in bone area, which is due to formation, and a progressive increase in medullary cavity area, which is due to resorption. Using this principle in conjunction with a tetracycline labeling procedure, it is possible to quantitate bone formation and resorption. Because tetracycline is only incorporated into sites where new bone is being formed, the volume of bone labeled with tetracycline during a known period of time is a measure of the bone formation rate.

The principle of the method used to measure the process of mineralization is shown schematically in Fig. 2. As illustrated, after osteoblasts form osteoid, which is unmineralized matrix, there is a lag period of about one day before mineralization is initiated at the mineralizing front and once initiated, mineral concentration increases until it reaches a maximum in mature bone. Thus, the process of bone mineralization can be divided into two components: first, the initiation of mineralization and second, the rate of mineralization once initiated. The initiation of mineralization was evaluated by determining the time between the formation of osteoid and the subsequent initiation of mineralization of this osteoid. This parameter, which has been termed the mineralization lag time, was calculated by dividing osteoid width by the linear rate of matrix formation, which is the width of new matrix formed per day. Mineral concentration, which was used to calculate the

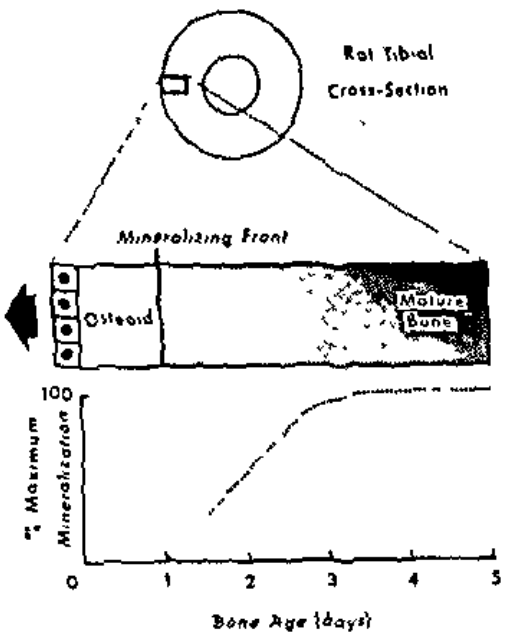


Fig 2. Schema of bone matrix formation and mineralization. The enlarged diagram of periosteal bone, as well as the graph below, illustrate the changes which occur in mineral concentration after matrix formation Bone age after matrix formation is on the abscissa which applies to both the graph and diagram The arrow indicates the direction of periosteal matrix formation and the squares represent osteoblasts Mineral concentration, as a percent of maximum in mature bone, is on the ordinant.

mineralization rate in young bone, was determined from measurements of refractive index, since the refractive index in bone varies linearly with the percent volume of hydroxyapatite (2).

MATERIALS AND METHODS

The protocols for the three experiments performed in the present study are given in Table 1. The objective of the first experiment was to measure the effects of fluoride on bone formation, mineralization and resorption. Although in this experiment periosteal matrix formation was 16% *more in* the fluoride treated than in the control group, the difference was not statistically significant Therefore, the second experiment was done to determine if administering fluoride for a longer *period of time would result in* a significant increase in formation. The third experiment was done to measure the net effects of long term fluoride administration on formation and resorption (*i.e*, *the effects of fluoride* on total area, medullary area, and bone area). Note that the third experiment is not *strictly comparable* to the other two experiments (Table 1). Despite these differences, the net effects of fluoride found in experiment 3 were consistent with the changes in the rates of periosteal bone formation and endosteal bone resorption found in experiments 1 and 2.

The protocol for the first experiment is shown diagrammatically in Fig 3. Forty male weanling SPRAGUE-DAWLEY rats were divided into four groups,

Table 1. Protocols for the three fluoride experiments

Experiment number	*Duration of treatment (days)*	*Age when fluoride was started (days)*	*Sex*	*Fluoride dose (ppm)**	*Bone measured*
1	*15*	*22*	male	0, 30, 100	tibia
2	35	22	male	0, 30, 100	tibia
3	113	21	female	0, 70	femur

* In drinking water.

each group having a mean weight of about 40 grams. At the start of the experimental period, one group was sacrificed for base line bone and serum measurements and the other three groups were sacrificed 15 days later. One of these three groups served as the control group, receiving tap water containing less than .01 ppm fluoride, and the other two groups served as the experimental groups, one receiving 30 ppm fluoride and the other 100 ppm fluoride in the drinking water. The rats in the basal group were given 20 mg/kg body weight of tetracycline i.v. 6 hours prior to sacrifice. At the same time that the rats in the basal group were sacrificed, the rats in the final groups (i.e., the control and two experimental groups) were started on daily i.p. injections of tetracycline which were continued until sacrifice on day 15 of the experimental period. The dose of tetracycline was 10 mg/kg body weight except for the first and last injections which were 20 mg/kg. In a previous study we showed that in rats so treated with tetracycline body weight gain is normal (5). Blood was obtained from all animals by cardiac puncture just prior to sacrifice. Serum calcium was measured by atomic absorption spectrometry (30) and serum phosphorus using an autoanalyzer (11).

The protocol for the second experiment (Table 1) was basically the same as that used for the first experiment. However, in this experiment tetracycline was given only during the last 8 days of the 35 day fluoride treatment period. Since the measuring period began after fluoride was started, it was necessary to have a basal group for each of the three final groups (i.e., for the control group and the groups given 30 and 100 ppm fluoride in the drinking water). The basal groups were sacrificed at the same time that the measuring period began in the final groups (i.e., on the 27th day of the experimental period).

After sacrifice, the femurs and tibias were removed and cleaned of soft tissue down to, but not including, the periosteum. Each entire left femur was ashed and the ash was dissolved in 5 N HCl for measurements of total calcium by atomic absorption spectrometry and of total fluoride by a specific

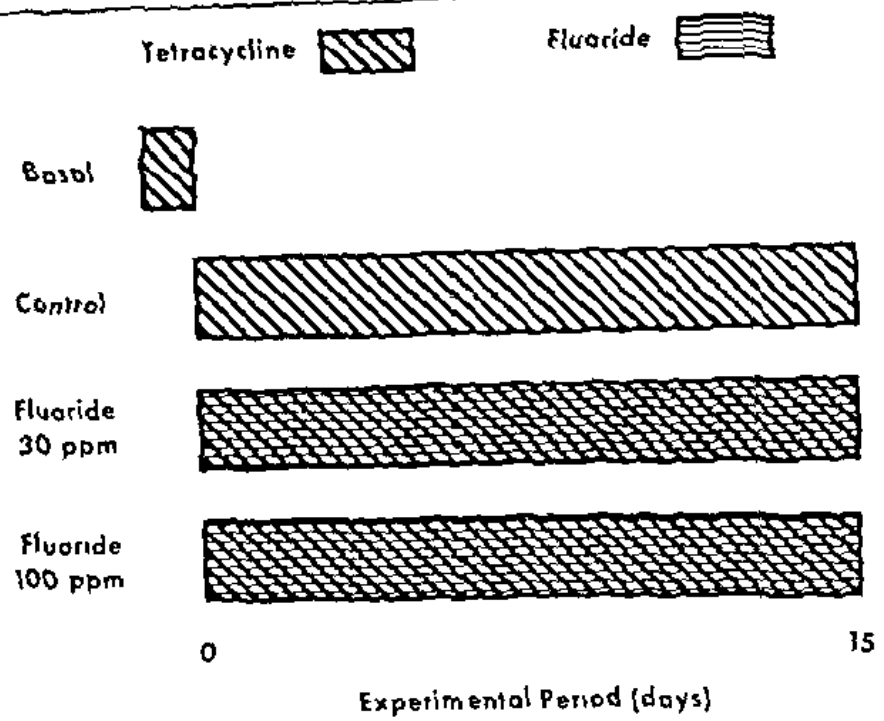


Fig 3 Experimental protocol for experiment 1 (15 days). The group designations are given on the left The fluoride concentration used to designate the two experimental groups represents that in the drinking water. The basal group received an i p injection of tetracycline 6 hours before sacrifice, whereas the three final groups received daily i p injections of tetracycline throughout the experimental period

ion electrode (26) Fluoride in ppm of bone ash was calculated for the total femur by assuming that calcium represented 38% of the ash content (16).

The quantitative histological measurements were made on the left tibias. Two consecutive transverse ground sections were prepared from each rat from the left tibial diaphysis proximal to the fibular junction. The first ground section was mounted unstained in Abopon and used for area, width and surface length measurements The entire area of the unstained section was photographed using tungsten and ultraviolet illumination. Enlarged prints with a final magnification of 73× were then made from each photomicrograph and from these, area measurements were made with a planimeter and surface length measurements were made with a map meter. At the endosteum, a forming surface was identified by the presence of osteoid and an appositional tetracycline label, and an actively resorbing surface was identified by the absence of such a label. That an unlabeled surface represents an actively resorbing surface was determined from a previous study in which we found that all of the endosteal surface that was not forming (i.e., where there was no appositional tetracycline label) displayed intense extracellular

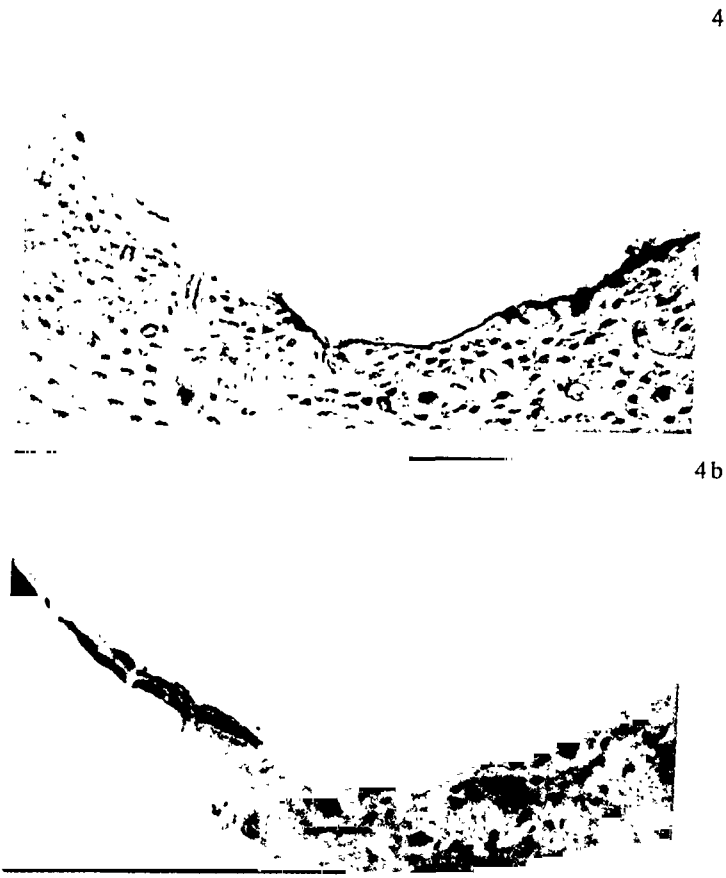


Fig. 4. a) Bright field photomicrograph and b) fluorescence photomicrograph of the same field of endosteal bone showing acid phosphatase activity (using α-naphthol phosphate as the substrate) along the resorbing surface on the left and a two day appositional tetracycline label along the forming surface on the right. Sites of acid phosphatase activity are red, whereas tetracycline fluorescence is yellow. (Original magnification 42×.)

acid phosphatase activity along the borders of HOWSHIP's lacunae (Fig. 4a and b). From this study we concluded that the absence of an appositional tetracycline label at the endosteum is indicative of active osteoclastic bone resorption at our sampling site (6, 29).

The second ground section was stained with a modified VON KOSSA procedure, counterstained with nuclear fast red, dehydrated in acetone, cleared in xylene and mounted in Fluormount for measurements of osteoid width and, in the basal groups, for measurements of the length of forming and

Table 2 Method and precision of the morphological measurements

Measurement	*Method*	*Precision**
Width		
Osteoid seam	filar micrometer	± 2–5%
6 hour label		
Area		
Total		
Medullary cavity	planimeter	± 1–4%
Tetracycline label		
Surface		
Periosteal		
Endosteal	map-meter	
Forming	or	± 1–6%
Resorbing	line sampling	

* Coefficient of variation.

resorbing surfaces, using the line sampling method, with a Zeiss No. II type integrating eyepiece. Osteoid width was measured with a filar micrometer every 60 degress around the periosteum and at four equidistant positions along the endosteum. The width of the six hour tetracycline label was measured in unstained ground sections from all rats in the basal groups using ultraviolet illumination and the same sampling procedure described above for osteoid In a separate experiment we found that the above method, because of the resulting dehydration, caused osteoid width to shrink 25.5%. On the other hand, we found no shrinkage of bone which was mounted in the water soluble mounting media, Abopon. Therefore, all osteoid width data given in the present study has been corrected for shrinkage so that the osteoid and bone masurements would correspond and both apply to the *in vivo* state.

Table 2 shows that measurements of width, area and surface length were generally made with a precision of ± 5% or less.

The maximum mineral concentration within the mineralizing front, as a percent of the maximum mineral concentration *in mature* bone, was estimated from refractive index (nD) measurements, since there is a direct linear relationship between the nD and the percent volume of hydroxyapatite (2). The mineralizing front is defined as the width of newly mineralized bone (adjacent to osteoid) which takes up an *in vivo tetracycline* label as the result of tetracycline diffusion into the mineral phase and not as a result of new bone apposition The nD of osteoid, the maximum nD in the mineralizing front, and the maximum nD in mature bone were measured at the edge of a bisected transverse ground section by means of the BECKE line procedure, using

monochromatic light (5460 Å) and a series of oils with nD-gradations of 0.002 (1).

In experiment 3 the control group, consisting of 11 female rats, was given tap water (< .01 ppm fluoride) to drink and the experimental group, consisting of 8 female rats, was given 70 ppm fluoride in the drinking water for 113 days beginning at 21 days of age. After sacrifice the femurs were bisected and ground sections were prepared from the mid-femoral diaphysis for measurements of total area, medullary area, and bone area using methods described for experiments 1 and 2.

Calculations

In a previous study we showed that in our sampling site about 90% of osteoblastic formation and osteoclastic resorption occurs at the periosteum and endosteum (5). For this and other reasons (5), all measurements were confined to the periosteum and endosteum in the present study.

Basal and final refer to parameters measured in the basal and final groups respectively. The initial parameters were also measured in the final group but refer to the periosteal values at the beginning rather than the end of the tetracycline labeling period; the inner border of the periosteal tetracycline label shows the position of the periosteal mineralizing front at the start of the labeling period. The total area is the area circumscribed by the periosteal edge of mineralized bone and thus includes the medullary area. Area and surface measurements on the prints and sections were made at the edge of mineralized bone at a resorbing surface, or on mineralized bone at a junction between labeled and unlabeled bone, or at the junction between mineralized bone and osteoid; therefore, osteoid tissue was not included in any of these measurements.

In all equations, the units are mm^2 for area, mm for length and width and days for time. Since the length of the tibial sampling site was more than 1 mm, the final rates were expressed as the volume of bone formed or resorbed per day, assuming that the length of the sample in each case was 1 mm.

In most calculations, only measured areas were used. However, for periosteal osteoid area and periosteal 6 hour tetracycline labeled area, the area was calculated from width and circumference measurements according to the formula for the area of an annulus,

$$A = W(L_i + \pi W) = W(L_o - \pi W), \qquad [1]$$

where A is area, W is width and L_i and L_o are the internal and external circumferences respectively. The accuracy of this approximation was tested

by comparing the total bone area calculated using the assumption that the periosteal circumference is a circle, with the measured total area. These calculated areas were found to be only 3% more than the measured area.

Since formation occurred on only a portion of the endosteal surface, the formula for the area of a sector of an annulus was used to calculate endosteal osteoid area and endosteal 6 hour tetracycline labeled area,

$$\text{Á} = \frac{\text{W L}_f}{\text{L}_i}(\text{L}_i + \pi\,\text{W}) = \frac{\text{W L}_f}{\text{L}_o}(\text{L}_o - \pi\,\text{W}), \qquad [2]$$

where Á is endosteal osteoid area or endosteal 6 hour tetracycline labeled area and L_f is the length of the forming surface.

The description of each parameter and the formulas used to calculate the parameters in this study are given below.

(1) Periosteal bone formation rate (R_{pbf}). In principle, the amount of periosteal bone formed per day was calculated by dividing the periosteal tetracycline labeled bone area by the length of the experimental period. The periosteal labeled area was obtained in the final groups by subtracting the initial total area (the area encompassed by the central border of the tetracycline label) from the final total area. However, the measured labeled area included the mineralizing front which existed at the beginning of the experimental period and was formed prior to the experimental period. To obtain the true amount of bone formed per day, the area corresponding to the first 6 hours of the final periosteal label (calculated from the initial periosteal circumference and the mean basal 6 hour label width) was subtracted from the final labeled area and the result was divided by the length of measuring period minus 6 hours,

$$R_{pbf} = \frac{A_{ft} - A_{it} - A_p}{T_f - T_b}, \qquad [3]$$

The endosteal bone formation rate was calculated in a similar manner in that the 6 hour labeled area was subtracted from the endosteal labeled area in the final groups and the result was divided by the length of the measuring period minus 6 hours. The 6 hour labeled endosteal area was obtained using [2], the formula for a sector of an annulus. The total bone formation rate was obtained by adding the periosteal and endosteal rates.

(2) Periosteal matrix formation rate (R_{pmf}). This includes the amount both of unmineralized matrix (osteoid) and of mineralized matrix formed at

the periosteum. The periosteal matrix formation rate was calculated by adding the increase in osteoid area per day to the area of bone formed per day. The increase in osteoid area per day was calculated by subtracting the initial osteoid area from the final total osteoid area and dividing by the length of the measuring period. The initial and final periosteal osteoid areas were calculated from osteoid width and the periosteal circumference using [1], the formula for the area of an annulus. This rate was calculated by the formula,

$$R_{pmr} = R_{pbr} + \frac{A_{of} - A_{oi}}{T_r}, \qquad [4]$$

where A_{of} is the final periosteal osteoid area and A_{oi} is the initial periosteal osteoid area.

The endosteal matrix formation rate was calculated in a similar manner except that osteoid area was obtained from [2], the formula for the area of a sector of an annulus, since formation does not occur around the entire endosteal circumference. The total matrix formation rate was obtained by adding the periosteal and endosteal rates.

(3) Periosteal bone apposition rate (R_{pba}). This is the linear rate of periosteal bone formation and was calculated by dividing the total periosteal area of bone formed per day by the mean of the initial and final periosteal circumferences using the formula,

$$R_{pba} = \frac{2\,(A_{rt} - A_{it} - A_p)}{(T_r - T_b)\,(L_f + L_i)}, \qquad [5]$$

where L_f is the length of final periosteal circumference and L_i is the length of initial periosteal circumference.

(4) Periosteal matrix apposition rate (R_{pma}). This is the mean width of matrix formed per day at the periosteum and takes into account both osteoid and mineralized matrix. It was calculated by the formula,

$$R_{pma} = R_{pba} + \frac{W_{of} - \overline{W}_{ob}}{T_r}, \qquad [6]$$

where W_{of} is the final periosteal osteoid width and $\overline{W}_{ob}$ is the mean basal periosteal osteoid width.

(5) Mineralization lag time (T_m). This is the time between the formation of osteoid and the subsequent onset of mineralization in this osteoid. It was calculated at the periosteum by dividing the mean osteoid width for the experimental period by the corresponding matrix apposition rate,

$$T_m = \frac{\overline{W}_{ob} + W_{of}}{2\,R_{pma}}, \qquad [7]$$

where R_{pma} is the matrix apposition rate at the periosteum.

(6) Mineralization rate (R_m). The mineralization rate is defined as the rate at which the concentration of mineral increases in the mineralizing front. To calculate this parameter, it is necessary to determine the maximum concentration of mineral in the mineralizing front. This was calculated from refractive index measurements using the formula,

$$C = \frac{nD_{mf} - nD_o}{nD_{max} - nD_o}, \qquad [8]$$

where C is the maximal mineral concentration in the mineralizing front, expressed as a fraction of the maximum mineral concentration in mature bone, nD_{mf} is the maximum refractive index in the mineralizing front, nD_{max} is the refractive index in mature bone and nD_o is the refractive index in osteoid. The time required to achieve this mineral concentration was calculated by dividing the width of the mineralizing front at the periosteum by the periosteal bone apposition rate. After an i.p. injection of tetracycline, the blood level is sufficiently high to label all bone formed for at least 6 hours (5). Therefore, since in the basal groups the 6 hour tetracycline label width included the mineralizing front plus 6 hours of apposition, the mineralizing front width was calculated by subtracting the width of bone formed in 6 hours from the 6 hour tetracycline label width. In the final groups, the mineralizing front could not be measured, because it could not be distinguished from that portion of the label resulting from apposition. Since in the second experiment (35 days) the osteoid width was increased to about the same extent in the basal and final fluoride treated groups, we assumed that the mineralizing front width was also constant during the measuring period (8 days) and that the basal mineralizing front width was equivalent to the mean width for the measuring period.

Although in previous studies it has been demonstrated that high doses of tetracycline inhibit mineralization (13), it is unlikely that tetracycline significantly altered the mineralization rates as determined by the method used in this study for two reasons. First, the dose of tetracycline used in this study was considerably less than that necessary to demonstrate inhibition of mineralization by microradiography (13). Second, the mineralization rate is inversely related to the distance that tetracycline diffuses into mineral which was laid down before tetracycline was administered.

The mineralization rate at the periosteum was calculated by the formula,

$$R_m = \frac{C\,R_{pbs}}{\overline{W}_{mfb}}, \qquad [9]$$

where $\overline{W}_{mfb}$ is the mean width of the mineralizing front of the basal group.

(9) Endosteal bone resorption rate (R_{ebr}). Since no resorption occurred at the periosteum, this represents the total resorption rate. In principle, the endosteal osteoclastic bone resorption rate was calculated by adding the difference in medullary area between the basal group and the final groups to the area of endosteal labeled bone and dividing the sum by the length of the experimental period. Since the basal medullary area did not contain the mineralizing front, it was necessary to subtract the basal endosteal mineralizing front area from the endosteal labeled area, to obtain the true change in medullary area due to resorption. In a previous study using this method for measuring bone resorption, we showed that none of the endosteal bone formed and labeled during the measuring period is resorbed before the end of this period even when the resorption rate is increased (6). It was calculated by the formula,

$$R_{ebr} = \frac{A_f + A_{el} - A_b - \bar{A}_{mf}}{T_f}, \qquad [10]$$

where A_f is the final medullary area, A_{el} is the endosteal labeled area, A_b is the basal medullary area, and $\bar{A}_{mf}$ is the mean basal endosteal mineralizing front area.

(10) Linear rate of endosteal bone resorption (R_{lbr}). This is the mean width of endosteal bone resorbed per day and is analogous to the periosteal bone apposition rate. It was calculated by dividing the area of bone resorbed per day by the mean of the basal and final endosteal resorbing surfaces by the formula,

$$R_{lbr} = \frac{2\,R_{ebr}}{\bar{L}_{rb} + L_{rf}}, \qquad [11]$$

where $\bar{L}_{rb}$ is the mean basal endosteal resorbing surface and L_{rf} is the final endosteal resorbing surface.

(11) Maximum bone stress (σ_{max}). This is the maximum stress which would be expected under normal conditions in our sampling site in the femoral diaphysis. Stress refers to the intermolecular resistance within bone as a result of the application of external force to bone from e.g., weight bearing and muscular action. It was calculated by the formula,

$$\sigma_{max} = \frac{(R^2 + r^2)\cos\alpha + 4\,L_b\,R\,\mathrm{Sin}\,\alpha}{\pi\,(R^4 - r^4)} \cdot WC, \qquad [12]$$

where R is the radius of the total area, r is the radius of the medullary area, α is the angle at the knee between a vertical line and the femur when the knee is flexed (assumed to be 75°), L_b is the length of the bisected femur,

W is the final body weight, and C is the maximum portion of the body weight supported by the left hind limb (assumed to be half of the body weight). This formula is based on the assumption that the femoral diaphysis in our sampling site is a hollow column.

Significance estimates were made using STUDENT'S *t* test on parameters calculated from the formulae given above. In order to estimate the variance of the measurement of bone resorption, it is necessary to determine the resorption rate for each rat individually. To do this it is necessary to know the initial medullary area at the beginning of the measuring period in the rats sacrificed at the end of this period. Since it was not technically possible to measure directly initial medullary area, the variance for bone resorption could not be estimated in the usual manner. However, if one makes the reasonable assumption that rats with a smaller medullary area have a lower bone resorption rate than those with a larger medullary area, the rats can be paired according to medullary size to calculate individual resorption rates. Thus, we ranked rats in the basal group and in the final groups in descending order according to the size of their medullary area and then subtracted the basal medullary area from the sum of the final medullary area and the endosteal labeled area of corresponding rank position to obtain individual bone resorption rates The relative variance for bone resorption obtained by this ranking procedure was still considerably higher than for any other morphological parameter calculated in the present study (Tables 4 and 5)

RESULTS

In experiments 1 (15 days) and 2 (35 days), fluoride administration did not affect either final body weight or serum calcium or phosphorus (Table 3). Neither was there a significant difference in final body weight in experiment 3 (113 days) between the control and fluoride treated groups (265 $\pm$ 26 gm vs 272 $\pm$ 35 gms)* Also shown in Table 3 is that the fluoride concentration in bone ash varies directly with the dose of fluoride and also with the length of time fluoride is administered.

The validity of our method for measuring bone formation and resorption depends on the demonstration that formation occurs continuously around the entire periosteum and that at the endosteum, bone surfaces that are actively involved in formation and resorption at the time of sampling can be accurately identified In experiment 1 (and also in experiment 2) bone formation as indicated by tetracycline fluorescence occurred around the entire periosteal circumference (Fig. 5). In addition, in previous studies we demon-

* Mean $\pm$ standard deviation.

Table 3. Effect of fluoride treatment in experiments 1 (15 days) and 2 (35 days) on final body weight, serum calcium and phosphorus, and fluoride concentration in the total femur

Experiment number	*Group*	*Final body weight (gm)*	*Serum calcium (mg/100 ml)*	*Serum phosphorus (mg/100 ml)*	*Femur fluoride concentration (ppm in ash)*
1	Control	144 ± 10*	10.1 ± 0.4	10.2 ± 0.6	83 ± 20
	30 ppm fluoride**	150 ± 8	10.2 ± 0.4	11.2 ± 0.8	1458 ± 182
	100 ppm fluoride	137 ± 9	9.9 ± 0.3	10.5 ± 0.9	5095 ± 1404
2	Control	182 ± 17	9.8 ± 0.3	9.9 ± 0.7	120 ± 53
	30 ppm fluoride	187 ± 12	9.8 ± 0.4	9.9 ± 0.5	2331 ± 424
	100 ppm fluoride	174 ± 11	9.7 ± 0.3	9.7 ± 0.6	6319 ± 975

* Mean ± standard deviation.
** Concentration of fluoride in drinking water.

Table 4 *Effect of 15 days of fluoride treatment (experiment 1) on bone formation, mineralization, and resorption*

	Control	*30 ppm fluoride**	*100 ppm fluoride*	*Control vs 100 ppm fluoride* % *change*	*P***
Periosteal formation (mm^3/day)					
a) bone	0 0727 ± 0 0145***	0 0760 ± 0 0079	0 0815 ± 0 0088	+ 12	> .1
b) matrix	0 0733 ± 0 0146	0 0779 ± 0 0081	0 0847 ± 0 0086	+ 16	< .06
Periosteal apposition (μ/day)					
a) bone	12 7 ± 2 4	13 1 ± 1 3	13 9 ± 1 2	+ 9	> .1
b) matrix	12.7 ± 2 4	13 3 ± 1 4	14 3 ± 1 2	+ 13	< .1
Mineralization lag time (days)	0.71 ± 0 13	0 77 ± 0 07	0 83 ± 0 10	+ 17	< 05
Endosteal bone resorption (mm^3/day)	0 0030 ± 0 0034	0 0099 ± 0 0031	0 0120 + 0 0049	+ 300	< .001
Linear rate of endosteal bone resorption (μ/day)	1.7 ± 1.9	5 1 ± 1 5	5 0 ± 2 1	+ 194	< .005

* Concentration of fluoride in drinking water
** Probability estimated by t test
*** Mean ± standard deviation.

Table 3. Effect of fluoride treatment in experiments 1 (15 days) and 2 (35 days) on final body weight, serum calcium and phosphorus, and fluoride concentration in the total femur

Experiment number	*Group*	*Final body weight (gm)*	*Serum calcium (mg/100 ml)*	*Serum phosphorus (mg/100 ml)*	*Femur fluoride concentration (ppm in ash)*
1	Control	144 ± 10*	10.1 ± 0.4	10.2 ± 0.6	83 ± 20
	30 ppm fluoride**	150 ± 8	10.2 ± 0.4	11.2 ± 0.8	1458 ± 182
	100 ppm fluoride	137 ± 9	9.9 ± 0.3	10.5 ± 0.9	5095 ± 1404
2	Control	182 ± 17	9.8 ± 0.3	9.9 ± 0.7	120 ± 53
	30 ppm fluoride	187 ± 12	9.8 ± 0.4	9.9 ± 0.5	2331 ± 424
	100 ppm fluoride	174 ± 11	9.7 ± 0.3	9.7 ± 0.6	6319 ± 975

* Mean ± standard deviation.
** Concentration of fluoride in drinking water.

Table 4. Effect of 15 days of fluoride treatment (experiment 1) on bone formation, mineralization, and resorption

	Control	30 ppm fluoride*	100 ppm fluoride	Control vs 100 ppm fluoride % change	P**
Periosteal formation (mm³/day)					
a) bone	0 0727 ± 0.0145***	0 0760 ± 0 0079	0 0815 ± 0 0088	+ 12	> .1
b) matrix	0.0733 ± 0.0146	0.0779 ± 0 0081	0 0847 ± 0 0086	+ 16	< .06
Periosteal apposition (μ/day)					
a) bone	12.7 ± 2.4	13 1 ± 1 3	13.9 ± 1.2	+ 9	> .1
b) matrix	12.7 ± 2 4	13 3 ± 1 4	14 3 ± 1 2	+ 13	< .1
Mineralization lag time (days)	0.71 ± 0 13	0 77 ± 0.07	0 83 ± 0.10	+ 17	< .05
Endosteal bone resorption (mm³/day)	0 0030 ± 0 0034	0 0099 ± 0 0031	0 0120 ± 0 0049	+ 300	< .001
Linear rate of endosteal bone resorption (μ/day)	1.7 ± 1 9	5 1 ± 1.5	5.0 ± 2.1	+ 194	< .005

* Concentration of fluoride in drinking water
** Probability estimated by t test.
*** Mean ± standard deviation.

Table 5. Effect of 35 days of fluoride treatment (experiment 2) on bone formation and mineralization

	Control	*30 ppm fluoride**	*100 ppm fluoride*	*Control vs 30 ppm fluoride* % *change*	*P***
Total formation (mm^3/day)					
a) bone	0.0687 ± 0.0097***	0.0782 ± 0.0140	0.0757 ± 0.0130	+ 14	< .05
b) matrix	0.0668 ± 0.0090	0.0779 ± 0.0132	0.0755 ± 0.0120	+ 17	< .02
Periosteal formation (mm^3/day)					
a) bone	0.0574 ± 0.0089	0.0671 ± 0.0122	0.0644 ± 0.0111	+ 17	< .02
b) matrix	0.0557 ± 0.0085	0.0664 ± 0.0112	0.0636 ± 0.0098	+ 19	< .01
Periosteal apposition (μ/day)					
a) bone	8.2 ± 1.1	9.6 ± 1.6	9.3 ± 1.4	+ 13	< .02
b) matrix	8.0 ± 1.1	9.4 ± 1.5	9.1 ± 1.3	+ 18	< .01
Mineralization lag time (days)	0.92 ± 0.15	0.95 ± 0.13	1.08 ± 0.14	+ 17****	< .025
Mineralization rate (% of maximum/hour)	2.00 ± 0.58		1.37 ± 0.38	— 32****	< .01

* Fluoride concentration in drinking water.
** Probability estimated by t test.
*** Mean ± standard deviation.
**** Control vs group given 100 ppm fluoride in the drinking water.

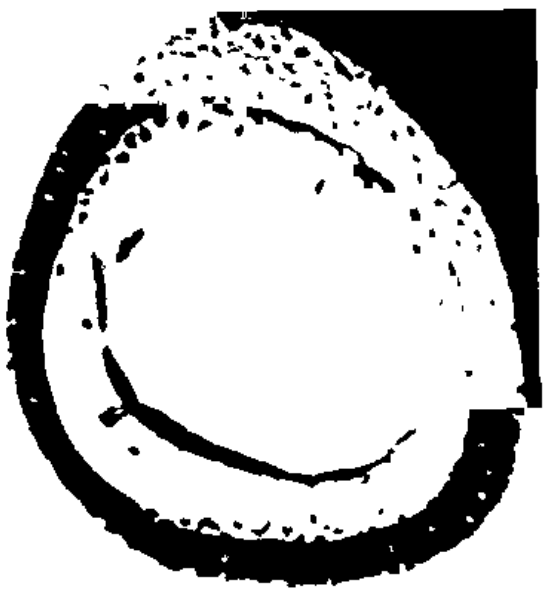


Fig 5. Fluorescence photomicrographs of tibial transverse sections from a) a control rat and b) a rat treated with 100 ppm fluoride in the drinking water in experiment 1 showing 15 day tetracycline labels around the entire periosteal circumference and along a portion of the endosteum. (Original magnification 10×.)

strated, using a double labeling procedure, that bone formation at the periosteum was continuous during the measuring period (5, 6). Bone formation occurred as well at the endosteum but was localized within a specific region (Fig. 5). Along the remainder of the endosteum, intense acid phosphatase staining (Fig. 4a) as well as the presence of HOWSHIP'S lacunae and osteoclasts indicated that osteoclastic bone resorption was occurring. Thus, the entire endosteal surface was involved in either formation or resorption (Figs. 4a and b).

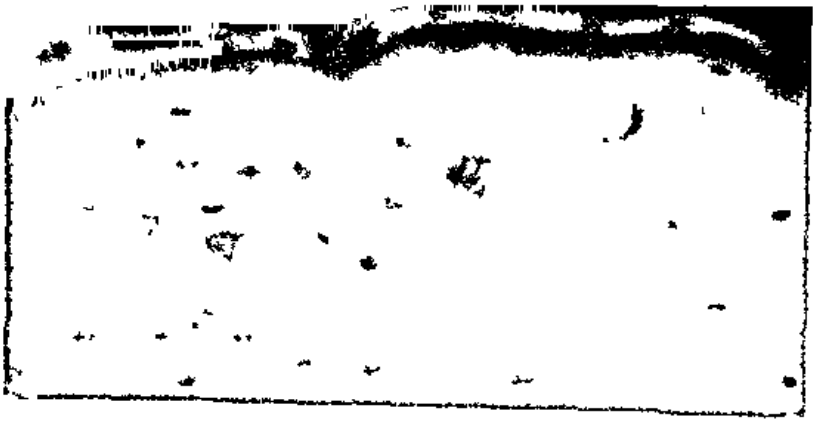
The effects of fluoride on formation, mineralization, and resorption in experiment 1 and on formation and mineralization in experiment 2 are given in Tables 4 and 5 respectively. In experiment 1, although the periosteal matrix formation rate was 16% more in the rats treated with 100 ppm fluoride in the drinking water than in controls, this difference was not statistically significant at the 0.05 probability level. Nevertheless, the final total area was significantly ($p < .025$) more in the group treated with 100 ppm fluoride in the drinking water than in the control group (3.351 ± 0.203 mm^2 vs 3.123 ± 0.194 mm^2), and since formation is continuous at the periosteum, this is indicative of a stimulation of periosteal bone formation by fluoride administration.

Table 5 shows that when rats were treated with 30 ppm fluoride in the drinking water for 35 days (experiment 2), there were significant increases in the periosteal bone and matrix formation rates. In addition, 30 ppm fluoride in the drinking water resulted in a significant increase in the periosteal matrix apposition rate and this was largely responsible for the increased periosteal matrix formation rate caused by fluoride treatment. However, an increase in apposition secondarily results in an increase in the periosteal circumference and thus an increase in the periosteal bone forming surface so that eventually both an increase in apposition and in forming surface contribute to the total increase in periosteal formation. The total (i.e., the endosteal plus the periosteal) rates of bone and matrix formation were also significantly increased in the rats treated with 30 ppm fluoride in the drinking water (Table 5).

Periosteal osteoid width was significantly more in rats treated with 100 ppm fluoride in the drinking water than in controls in experiment 1 (Figs. 6a and b and 7) and also in experiment 2. The increase in periosteal osteoid width was sufficiently more than the increase in periosteal matrix apposition in both experiments 1 and 2 so as to result in a prolongation of the mineralization lag time (i.e., an increase in the time between osteoid formation and the onset of mineralization) (Tables 4 and 5).

The 6 hour periosteal tetracycline label width, from which the amount of bone apposition occurring in 6 hours was subtracted to obtain the mineraliz-

6a



6b

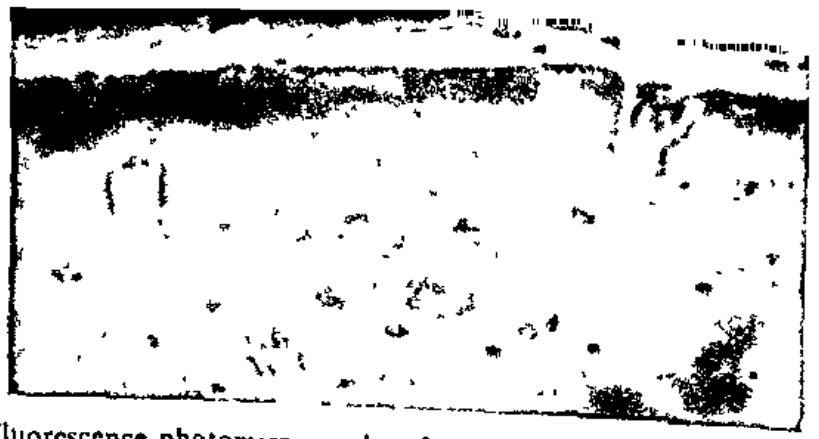


Fig 6 Fluorescence photomicrographs of periosteal osteoid in experiment 1 (15 days) from a) a control rat and b) a rat treated with 100 ppm fluoride in the drinking water The osteoblasts are stained red, the mineralizing front brown, and the intervening osteoid yellow (Original magnification 101 ×.)

in experiment 2 (Fig. 7) The maximum mineral concentration in the mineralizing front was estimated from refractive index (nD) measurements In both the control and experimental group, the nD of osteoid was 1.533, the maximum nD of the mineralizing front was 1.536, and the maximum nD in mature bone was 1.548. Based on the fact that there is a linear relationship between nD and degree of mineralization (2), the maximum mineral con-

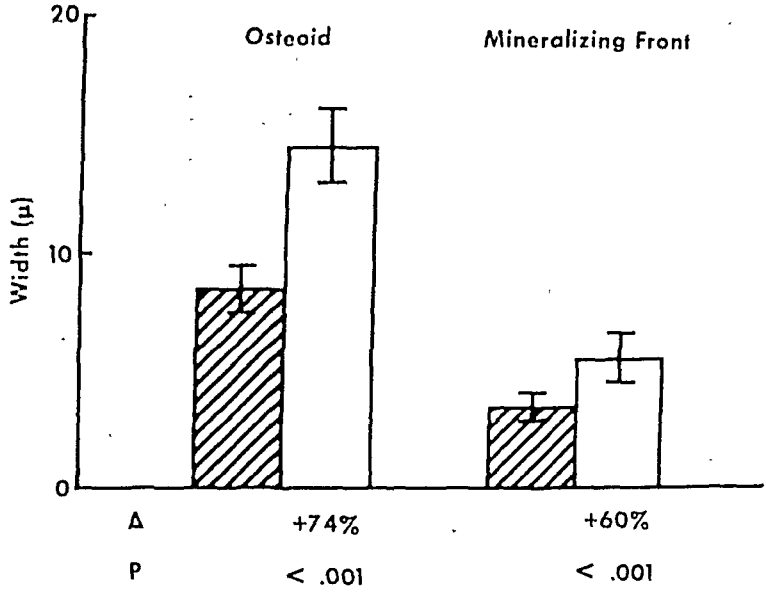


Fig. 7. Bar graphs of osteoid width in experiment 1 (15 days) and mineralizing front width in experiment 2 on the 27th day of the experimental period. The hatched bars represent the control groups and the plain bars represent the groups given 100 ppm fluoride in the drinking water.

centration in the mineralizing front in the control and experimental groups was 20% of the maximum mineral concentration in mature bone. Since the maximum mineral concentration was the same for the control and experimental groups, the mineralization rate represents the rate at which matrix mineralizes between the time mineralization is initiated in osteoid and when it reaches 20% of full mineralization.

As shown in Table 5, 100 ppm fluoride in the drinking water significantly decreased the rate of mineralization in young bone. It may be noted that in young bone there is an inverse relationship between the distance that tetracycline diffuses and the mineralization rate. The effects of 100 ppm fluoride in the drinking water on the mineralization lag time and on the mineralization rate in experiment 2 are shown graphically in Fig. 9. Since we measured mineral concentration at only one point in time, the mineralization rate between zero and 20% may not be constant. Nevertheless, the time required to reach 20% of maximum mineralization was significantly ($p < .01$) more in the fluoride treated than in the control group. Furthermore, we have recently demonstrated by a direct method, electron probe microanalysis, that the mineralization rate between 0 and about 90% of maximum is fairly constant, as illustrated in Fig. 2. In order to further establish that fluoride

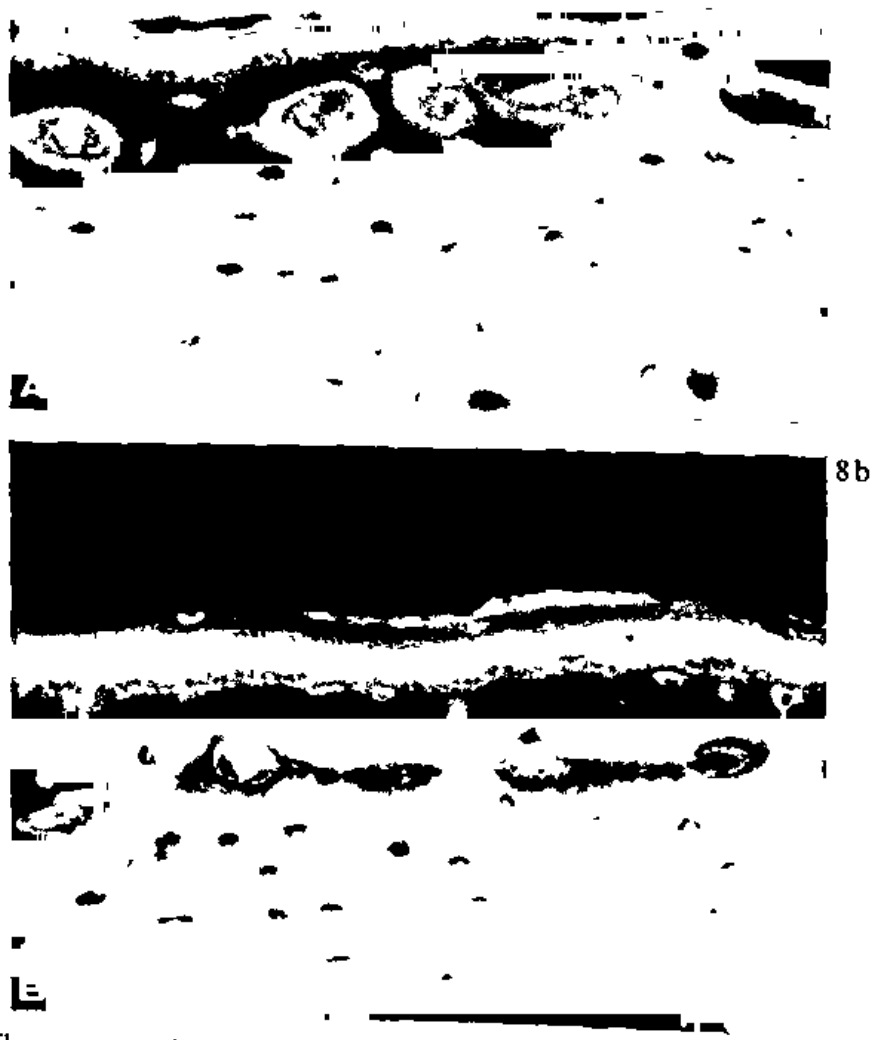


Fig 8 Fluorescence photomicrographs of 6 hour periosteal tetracycline labels in experiment 2 on the 27th day of the experimental period *from* a) a control rat and b) a rat given 100 ppm fluoride in the drinking water. (Original magnification 101 ×.)

administration decreases the rate of mineralization in young bone, an additional experiment was performed using 125 ppm fluoride in the drinking water* for 15 days In this experiment, the mineralization rate, expressed as a percent of maximum per hour, was 1.70 ± 0.28 for the controls compared with 0 86 ± 0.22 for the fluoride treated group. Thus, this dose of fluoride caused a marked (52%) reduction in the mineralization rate and this was statistically significant, $p < .001$.

* At this dosage, fluoride decreased body weight gain

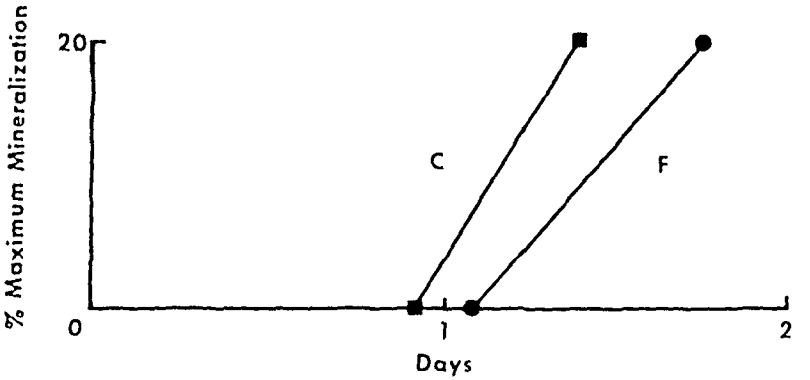


Fig. 9. Mineralization lag time and mineralization rate in experiment 2 (35 days). C represents the control group and F represents the group given 100 ppm fluoride in the drinking water. Mineral concentration is plotted as a function of time after periosteal matrix formation. The mineralization lag time, as indicated by the points on the abscissa, is the time between osteoid formation (zero time) and the onset of mineralization. The lines between 0 and 20% of maximum mineralization represent the mineralization rates.

In experiment 1, fluoride at both dosage levels caused a marked increase in the endosteal osteoclastic bone resorption rate (Table 4). In addition, the final medullary area was significantly ($p < .05$) more in a group treated with 100 ppm fluoride in the drinking water than in the control group (1.034 ± 0.140 mm^2 vs. 0.899 ± 0.115 mm^2) as would be expected from the increased endosteal bone resorption rate. In rats treated with 100 ppm fluoride in the drinking water compared with controls, there was also a significant increase in the linear rate of bone resorption (Table 4) and as well, a significant ($p < .001$) increase in the length of the endosteal resorbing surface ($2.303 \pm 0{,}683$ mm vs $1.174 \pm 0{,}376$ mm) as illustrated in Fig. 5 (also see Fig. 4). This absolute increase in resorbing surface may have arisen in part because the total endosteal surface was increased. However, in addition, there was a significant ($p < .005$) increase in the percentage of resorbing surface in the fluoride treated compared with the control group ($58 \pm 15\%$ vs $32 \pm 10\%$) and thus a significant ($p < .001$) reduction in the length of the endosteal forming surface in the fluoride compared with the control group (1.678 ± 0.609 mm vs 2.497 ± 0.459 mm). In contrast, although there was a significant increase in the linear rate of bone resorption in the group treated with 30 ppm fluoride in the drinking water (Table 4), the percentage of resorbing surface was not significantly increased in this group. No attempt was made to measure the bone resorption rate in experiment 2, because normally the resorption rate varies inversely with age in the sampling site

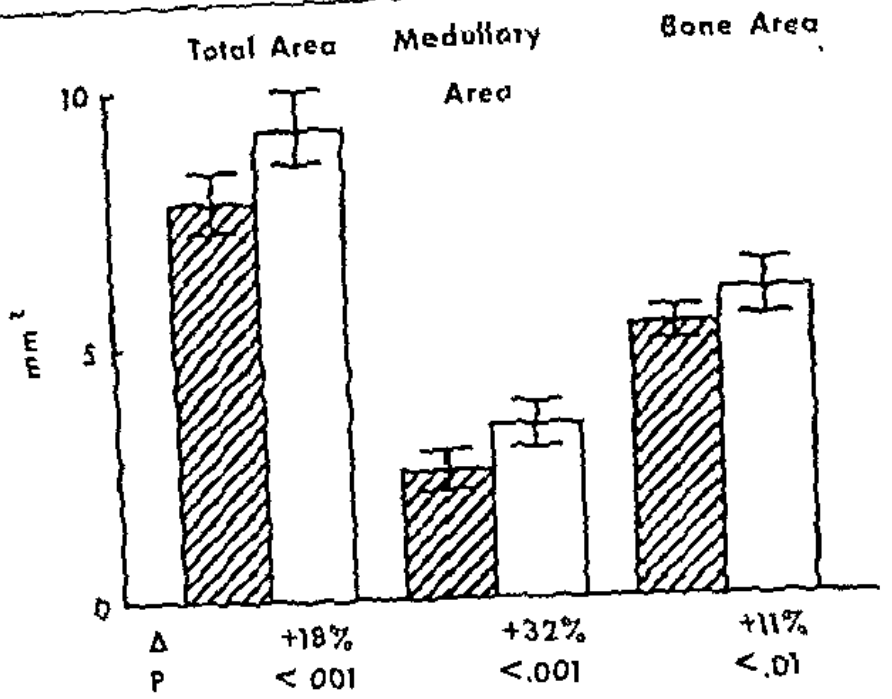


Fig. 10 Effects of fluoride on total area, medullary area, and bone area in experiment 3 (113 days) *The hatched bars represent the control groups* and the plain bars represent the groups given 70 ppm fluoride in the drinking water

used and consequently, the optimum time to measure endosteal bone resorption is in young rats

In experiment 3, there were *significant* increases in total area, medullary area, and bone area in rats treated with 70 ppm fluoride in the drinking water for 113 days as compared with controls (Fig 10). The magnitude of the increases in total area and medullary area was such that cortical thickness was similar in the control and experimental groups (0.677 ± 0.030 mm vs 0 676 ± 0 050 mm) Thus, the increase in bone area resulted because there was an increase in the periosteal perimeter. Because total area and medullary area are important determinants of the mechanical properties of a tubular bone, maximum stress in the femoral sampling site was calculated and the results are shown in Fig. 11. As illustrated, a significant decrease in maximum bone stress was found in the fluoride treated group. Thus, if bone quality, with respect to mechanical performance, were the same in the two groups, one could conclude that the bones of the fluoride treated group were stronger than those of the control group. However, we found that in the fluoride treated group, maximum birefringence was substantially diminished compared with the control group (Figs 12a and b). In contrast, collagen birefringence was only detectably diminished after 15 days of fluoride treat-

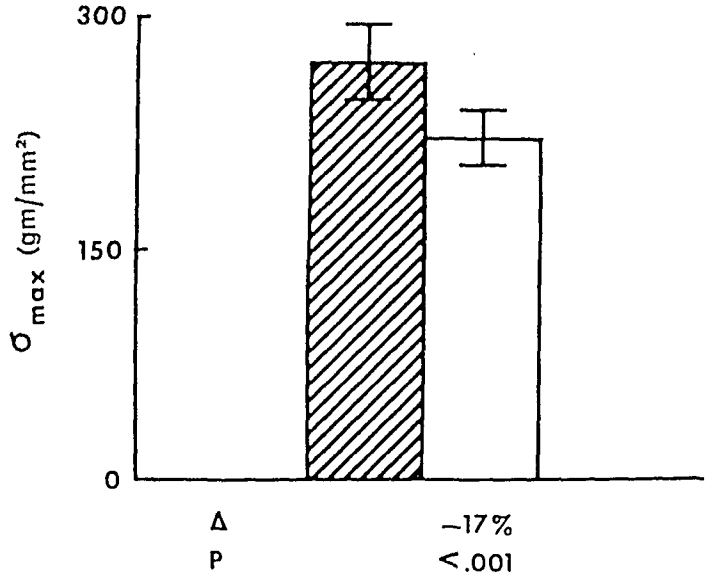


Fig. 11. Maximum bone stress (σ_{max}) in experiment 3 (113 days). The hatched bar represents the control group and the plain bar represents the group given 70 ppm fluoride in the drinking water. σ_{max} is the maximum stress which would be expected under normal conditions in our sampling site in the femoral diaphysis.

ment in experiment 1. Whether or not this diminished birefringence is indicative of a change in mechanical performance remains to be determined.

DISCUSSION

The methods used in the present study for measuring osteoblastic bone formation, mineralization and osteoclastic bone resorption have a number of advantages over existing methods as well as some limitations* (6). With respect to bone turnover, the present system was developed to determine the effects of an experimental condition on the two basic elements which determine the rate of formation and also the rate of resorption: (1) the number of cells involved in formation and resorption and (2) the effective functional

* All histological methods, including the one used in this work, for measuring the bone formation rate, measure the volume of bone formed and not the amount of mineral deposited in bone during the process of osteoblastic bone formation and mineralization. Therefore, since the mineralization rate is not invariant (as demonstrated in the present study), it is theoretically possible to have a change in volume of bone formed without a change in the amount of mineral deposited and vice versa. This disadvantage can be eliminated by simultaneous measurements of the volume of bone formed and the concentration of mineral within this volume.

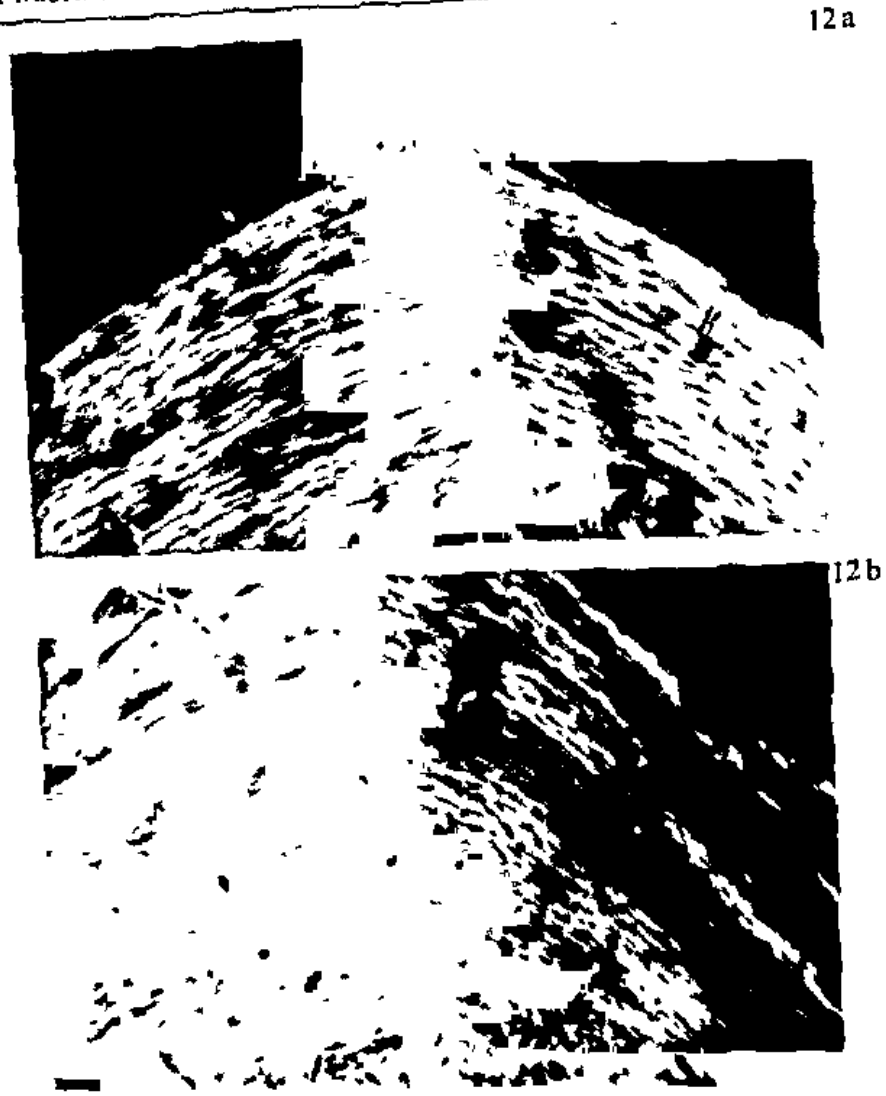


Fig 12. Photomicrographs showing collagen birefringence in the periosteal region of mineralized bone sections of the femoral diaphysis from a) a control rat and b) a rat given 70 ppm fluoride in the drinking water in experiment 3 (113 days). The photographs were taken using polarized light and, in order to avoid a difference in birefringence due to rotation, a first-order red compensator. Both blue and orange areas represent birefringence and in these areas the greater the brightness the greater the birefringence (Original magnification 101 ×)

activity of these cells The lengths of actively forming and resorbing surfaces were assumed to be a function of the number of osteoblasts and osteoclasts respectively, and the functional activities of osteoblasts and osteoclasts were evaluated from measurements of the linear rates of matrix formation and bone resorption respectively.

The quantitative data obtained in this study establish that fluoride administration has marked effects on bone metabolism. Three major effects of fluoride on bone were found: (1) an increase in periosteal matrix and bone

formation, (2) an inhibition of the process of mineralization at the periosteum, and (3) an increase in endosteal bone resorption (Tables 4 and 5). The increase in periosteal matrix formation resulted primarily because of increased osteoblastic synthetic activity per unit of forming surface as indicated by the increased periosteal matrix apposition rate. The inhibition of mineralization occurred because fluoride delayed the initiation of mineralization in osteoid (i.e., fluoride prolonged the mineralization lag time) and also decreased the rate of mineralization in young bone. In previous studies, vitamin D deficiency (6) and calcium deficiency (27) were also found to inhibit these two processes of mineralization; however, in both instances hypocalcemia was present, whereas in this study fluoride inhibited mineralization without affecting the serum levels of either calcium or phosphorus (Table 3).

The increased endosteal bone resorption in the group treated with 100 ppm fluoride in the drinking water in experiment 1 (15 days) resulted from an enhancement of two aspects of the process of bone resorption, First, the resorptive activity of osteoclasts per unit of resorbing surface was increased as evidenced by the increased linear rate of bone resorption. Second, the percentage of the endosteal surface involved in resorption was increased suggesting that the total number of osteoclasts was increased (i.e., that the differentiation of osteoprogenitor cells to osteoclasts was stimulated). It should be emphasized that since fluoride is mainly deposited in young bone (21; BAUD AND BANG, p. 27) and since none of the bone formed during fluoride administration was resorbed by osteoclasts in our sampling site (Fig. 5), the ability of osteoclasts to resorb bone containing a high concentration of relatively insoluble (7) fluorapatite was not tested in this study.

In terms of percent change from control values, fluoride increased periosteal formation much less than endosteal resorption; however, formation is an order of magnitude greater than resorption in the diaphysis of a growing rat. Consequently, a relatively small change in formation will have a substantial effect on the net amount of bone accumulated per day. Accordingly, fluoride increased bone area as well as total area (the area encompassed by the periosteal perimeter) and medullary area. Thus, the long term effect of fluoride administration on diaphyseal bone is a generalized increase in cross-sectional dimensions (Fig. 10). These changes appear to be a characteristic effect of long term fluoride administration, since an increase in the external diameter of bone accompanied by an enlargement of the medullary cavity has been found in both cattle (15, 19) and man (25) after long term exposure to fluoride.

The results of the present study are similar to the majority of our previous

findings in bone biopsies from patients treated with fluoride (3). However, two differences in the results of these two studies deserve comment. First, using the tetracycline labeling technique, we previously concluded that the linear rate of bone formation was decreased in osteoporotic patients treated with large doses of fluoride. As demonstrated in the present study and in our previous study, fluoride decreases the mineralization rate and as a result tetracycline diffuses into recently mineralized bone further than normal. This change compromises the accuracy of the measurement of bone apposition in biopsies but was corrected for in the present study, and consequently it is possible that our previous observation in humans on this parameter was in error. Second, although we found an increase in the amount of surface involved in osteoclastic resorption in humans, changes in the cytologic features of osteoclasts suggested that the rate of bone resorption might still be decreased. In view of the results of this study and those of others (15), it is probable that osteoclastic bone resorption is increased in humans treated with fluoride.

Although the oral dose of fluoride on a body weight basis was higher in this study than in our previous study in humans, the concentration of fluoride in bone was similar in the two studies. Since rats of the age used in this study drink about 10 ml of water per day, the daily dose of fluoride was about 3 mg/kg body weigth for the group given 30 ppm in the drinking water and 10 mg/kg body weight in the group given 100 ppm in the drinking water. In contrast, the dose of fluoride used to treat patients with osteoporosis was about 0.5–1.0 mg/kg body weight. In experiment 1 (15 days) there were 1458 ppm fluoride in the bone ash in the group given 30 ppm fluoride in the drinking water and 5095 ppm fluoride in the bone ash in the group given 100 ppm fluoride in the drinking water. Similarly, the concentration of fluoride in bone ash ranged between 3,000 and 7,000 ppm in osteoporotic patients given high doses of fluoride (about 0.5–1.0 mg/kg body weight) for one to two years (12). Thus, the concentration of fluoride in bone ash was similar in experiment 1 and in patients treated with high doses of fluoride for 1 to 2 years.

amount of bone formed during fluoride administration in humans given fluoride for 1 to 2 years was considerably less than 38% of the total amount of bone in the iliac crest biopsy used for chemical analysis. Since the majority of fluoride uptake in bone occurs in areas of new bone formation (21), it follows from the above data that the concentration of fluoride in the bone formed during fluoride therapy was at least as high in humans in our previous study as in the rats in the present study in experiment 1. Therefore, it seems likely that the effective dose of fluoride used in the present study and that used in a previous study in humans are not as strikingly different as suggested from the dose per body weight comparison. Nevertheless, factors in addition to the concentration of fluoride in bone may influence the effect of fluoride on bone metabolism. For example, the sentitivity of bone metabolism to fluoride may differ in growing rats and adult humans.

It has been suggested (15) that the fluoride-induced increase in periosteal bone formation is compensatory to poor bone mechanical performance which occurs after long term exposure to high doses of fluoride (22, 25). Because we found that the medullary area was increased during fluoride treatment, one might suppose that this change resulted in an increase in bone stress which in turn caused a stimulation of periosteal bone formation. However, bone stress was decreased (Fig. 11) rather than increased and therefore, on a mechanical basis the increase in medullary area could not have been responsible for the increased periosteal bone formation. Bone stress, as calculated in this study, takes into account the amount and distribution of bone mass but not the quality of the material composing bone mass. We evaluated one aspect of bone quality, collagen birefringence, which is primarily determined by collagen bundle orientation. We found that although collagen birefringence was considerably diminished after 113 days of fluoride treatment, it was only detectably diminished after 15 days of fluoride treatment. Thus, since the magnitude of the change in collagen birefringence appeared to be similar to that in periosteal bone formation after 15 days of fluoride treatment, it is possible that this change in bone quality contributed to the increased periosteal bone formation. However, we previously found that the length of the bone forming surface was substantially increased after only a few weeks of fluoride administration in adult humans (3), and it is highly unlikely that the mechanical performance of bone is sufficiently impaired in such a short time period to account for this finding. Thus, although the mechanical performance of bone is impaired after long term fluoride exposure, it seems unlikely that a change in mechanical performance is entirely responsible for the early effects of fluoride on bone formation, at least in humans.

The results of a number of studies suggest that fluoride administration results in secondary hyperparathyroidism. Despite the fact that RAISZ and TAVES concluded from indirect methods that parathyroid gland function was normal in rats treated for 6 to 12 weeks with large doses of fluoride (23), YATES *et al.* demonstrated that parathyroid hormone secretion in rats was probably increased when fluoride was given by lavage (31). NICHOLS *et al.* found that metabolic changes in bone biopsies from humans treated with fluoride were similar to those found in biopsies from patients with primary hyperparathyroidism (20). In addition, we previously noted that the histo-

measured by radioimmunoassay was increased as much as five fold in sheep treated with fluoride for 1 week (10). Thus, the bulk of evidence suggests that fluoride administration causes secondary hyperparathyroidism

In the present study secondary hyperparathyroidism would be a reasonable explanation for the observed increases in endosteal bone resorption, endosteal resorbing surface, and the linear rate of bone resorption (27). Furthermore, we found that when thyroparathyroidectomized rats were given parathyroid extract there was a significant increase in periosteal osteoid width (unpublished data), a change which is the same as that produced by fluoride in the present study. However, the results of the present study and those of the experiment using parathyroid extract are not strictly comparable because in the latter experiment hypercalcemia was present. The diminished collagen birefringence observed in the present study and previously found in endemic osteofluorosis (3) has also been observed in patients with renal osteodystrophy where the serum levels of parathyroid hormone are markedly increased (18) and also in primary hyperparathyroidism (unpublished observations).

The foregoing observations indicate that there are a number of similarities between the effects of excess parathyroid hormone and the administration of fluoride on bone. On the other hand, it is unlikely that all of the bone changes observed in this study were the result of secondary hyperparathyroidism For example, the generalized increase in cross-sectional bone dimensions as a result of fluoride administration is not characteristic of long standing hyperparathyroidism. In addition, it is possible that fluoride directly affects bone by inhibiting enzyme activity. We have shown that extracellular enzyme activities exist in the region where mineralization is initiated (29) and these enzyme activities may play a role in both the initiation and the rate of mineralization. Thus far, acid phosphatase and esterase have

been found in this region (29) and both enzymes are known to be inhibited by fluoride *in vitro* (14).

Although the mechanism whereby fluoride administration results in an increase in the secretion of endogenous parathyroid hormone is not entirely clear, the latter probably results from an effect of fluoride on bone mineral transfer rather than from a direct effect of fluoride on the parathyroid glands (31). A net decrease in mineral transfer from bone to blood and thus a compensatory increase in parathyroid hormone secretion could result from any one or a combination of the following: (1) the formation of an increased volume of mineralized bone in conjunction with osteoblastic matrix formation, (2) an increased rate of mineralization in this volume of bone, (3) decreased osteoclastic resorption, (4) decreased osteocytic resorption, and (5) a decreased level at which calcium in bone equilibrates with that in blood. The results of the present study rule out the second and third mechanisms and make the first mechanism theoretically tenable. The fourth mechanism cannot be ruled out because fluoride could decrease osteocytic resorption as either a result of the formation of relatively insoluble fluorapatite in the perilacunar mineral phase* or as a result of cell injury which we previously found to occur during fluoride administration. On the other hand, at least during long term fluoride administration, there appeared to be an increase in the number of resorbing osteocytes (3). Evidence to support the fifth mechanism was reported by YATES *et al.* (31). They suggest that the decreased solubility of bone salt after the incorporation of fluoride ion decreases the level at which bone and blood calcium equilibrate and as a result there is a compensatory increase in endogenous parathyroid hormone secretion. However, their data do not clearly rule out either the first or the fourth mechanisms cited above. Furthermore, the changes they observed were obtained when fluoride was administered by a lavage but not when it was given orally as in the present study. In conclusion, increased parathyroid hormone secretion during fluoride administration could result from the formation of an increased volume of mineralized bone, impaired osteocytic resorption, or a decreased level at which calcium in bone equilibrates with that in serum.

An alternate possible mechanism for the observed increase in osteoclastic resorption is that, instead of increasing parathyroid hormone secretion, fluoride stimulates adenyl cyclase activity in bone and this results in an increased bone concentration of cyclic AMP which is believed to be a mediator of the effect of parathyroid hormone on bone (9, 28). However, the studies

* Fluoride could have an early effect on osteocytic resorption because perilacunar mineral turnover is probably relatively rapid (4).

of CHASE and AURBACH indicate that the dose of fluoride necessary to stimulate adenyl cyclase in bone is too high to be compatible with cell viability (8).

SUMMARY

The processes of bone formation, mineralization, and resorption in the diaphysis were studied, using quantitative histological methods, in rats treated with 30 to 100 ppm fluoride in the drinking water for from 15 to 113 days. The concentration of fluoride in the ash of bone formed during fluoride administration was not more and probably less in this study than that previously found in osteoporotic patients treated with high doses of fluoride for 1 to 2 years. Three major effects of fluoride on bone were found: (1) an increase in periosteal bone formation, (2) an inhibition of mineralization in young bone, and (3) an increase in endosteal bone resorption. The long term (113 days) effects of fluoride on formation and resorption in diaphyseal bone were such that there was a generalized increase in cross-sectional dimensions (i.e., total area, medullary area, and bone area were all increased). These quantitative histological findings in fluoride treated rats are, for the most part, similar to the changes we previously observed in osteoporotic patients treated with high doses of fluoride for 1 to 2 years.

Although the cause of the increased periosteal bone formation was not established, it was not secondary to the enlargement of the medullary cavity on a mechanical basis because we found that bone stress was decreased after fluoride treatment. Fluoride delayed the onset of mineralization and also decreased the mineralization rate in young bone without altering the serum levels of calcium or phosphorus. Thus this study clearly demonstrates that inhibition of mineralization can occur without a reduction in either the serum calcium, phosphorus or calcium phosphorus product. The increased endosteal bone resorption resulted from (1) an increase in the resorptive activity of osteoclasts per unit of resorbing surface and (2) an increase in the percent

REFERENCES

1 ALLEN, R. M.: Practical refractometry by means of the microscope. R. P. Cargille Laboratories, Inc., Cedar Grove, New York (1962).
2 ASCENZI, A., and FABRY, C.: Technique for dissection and measurement of refractive index of osteones. J. Biophys. Cytol. *6*, 139 (1959).
3 BAYLINK, D., and BERNSTEIN, D. S.: The effects of fluoride therapy on metabolic bone disease. Clin. Orthop. *55*, 51 (1967).
4 BAYLINK, D., MOREY, E., and RICH, C.: Effect of calcitonin on osteocyte mineral transfer in the rat, in Parathyroid hormone and thyrocalcitonin (Calcitonin), New York, Excerpta Medica Foundation (1968), pp. 196–197.
5 BAYLINK, D., MOREY, E., and RICH, C.: Effect of calcitonin on the rates of bone formation and resorption in the rat. Endocrinology *84*, 261 (1969).
6 BAYLINK, D., STAUFFER, M., WERGEDAL, J., and RICH, C.: Formation, mineralization and resorption of bone in the vitamin D deficient rat. Manuscript submitted for publication (1969).
7 BELLIN, J., and LASZLO, D.: Metabolism and removal of Ca^{45} in man. Science *117*, 331 (1953).
8 CHASE, L. R., and AURBACH, G. D.: Personal communication.
9 CHASE, L. R., and AURBACH, G. D.: Parathyroid function and the renal excretion of 3′5′-adenylic acid. Physiology *58*, 518 (1967).
10 FACCINI, J. M., and CARE, A. D.: Effect of sodium fluoride on the ultrastructure of the parathyroid glands of the sheep. Nature *207*, 1399 (1965).
11 FISKE, C. H., and SUBBAROW, H. Y.: The colorimetric determination of phosphorus. J. Biol. Chem. *66*, 375 (1925).
12 GRON, P., MCCANN, H. G., and BERNSTEIN, D.: Effect of fluoride on human osteoporotic bone mineral. J. Bone Joint Surg. *48*, 892 (1966).
13 HARRIS, W. H., LAVORGNA, J., HAMBLEN, D. L., and HAYWOOD, E. A.: The inhibition of ossification in vivo. Clin. Orthop. *61*, 52 (1968).
14 HODGE, H. C., and SMITH, F. A.: Effects of fluoride on enzyme systems, in: Fluorine Chemistry, vol. IV, New York, Academic Press (1965), pp. 176–189.
15 JOHNSON, L. C.: Histogenesis and mechanisms in the development of osteofluorosis, in: Fluorine Chemistry, vol. IV, New York, Academic Press (1965), pp. 424–441.
16 LIKINS, R. C., PIEZ, K. A., and KUNDE, M. L.: Mineralization of turkey leg tendon. III. Chemical nature of the protein and mineral phases, in: Biological Systems, Washington, D.C., American Association for the Advancement of Science (1960), pp. 143–149.
17 LUTWAK, L., and WHEDON, G. D.: Osteoporosis. DM (April 1969).
18 MELICK, R., and MARTIN, T. J.: Immunoassay of parathyroid hormone in human plasma, in: Parathyroid hormone and thyrocalcitonin (Calcitonin), New York, Excerpta Medica Foundation (1968), pp. 440–441.
19 MILLER, G. W., and SHUPE, J. L.: Alkaline bone phosphatase activity as related to fluoride ingestion by dairy cattle. Amer. J. Vet. Res. *23*, 24 (1962).
20 NICHOLS, G., jr., FLANAGAN, B., and WOODS, J. F.: Parathyroid influences on bone biosynthetic mechanisms, in: The Parathyroid Glands: Ultrastructure, secretion and function. Chicago, Univ. Chicago Press (1965), pp. 243–260.

21 PERKINSON, J. D., WHITNEY, I. B., MONROE, R. A., LOTZ, W. E., and COMAR, C. L.: Metabolism of F^{18} in domestic animals. Amer. J. Physiol. *182*, 383 (1955).
22 PHILLIPS, P. H., GREENWOOD, D. A., HOBBS, C. S., HUFFMAN, C. F., and SPENCER, G. R.: The fluorosis problem in livestock production. Natl. Acad. Sci. – Natl. Res. Council Publ. No. 824 (1960).
23 RAISZ, L. G., and TAVES, D. R.: The effect of fluoride on parathyroid function and responsiveness in the rat. Calc. Tiss. Res. *1*, 219 (1967).
24 RICH, C., and IVANOVICH, P.: Response to sodium fluoride in severe primary osteoporosis. Ann. Intern. Med. *63*, 1070 (1965).
25 ROHOLM, K.: Fluoride intoxication. London, H. K. Lewis (1937).
26 SINGER, L., and ARMSTRONG, W. D.: Determination of fluoride in bone with the fluoride electrode. Anal. Chem. *40*, 613 (1968).
27 STAUFFER, M., BAYLINK, D., WERGEDAL, J., and RICH, C.: The effect of calcium deficiency on bone formation, mineralization and resorption in the rat. Manuscript in preparation.
28 VAES, G.: Parathyroid hormone-like action of N^6-2′-O-dibutyryladenosine-3′5′ (cyclic)-monophosphate on bone explants in tissue culture. Nature *219*, 939 (1968).
29 WERGEDAL, J. E., and BAYLINK, D. J.: Distribution of acid and alkaline phosphatase activity in the rat tibial diaphysis. Histochem. Cytochem., in press (1969).
30 WILLIS, J. B.: The determination of metals in blood serum by atomic absorption

The action of fluoride on bone*

C. Rich and E. Feist

We shall deal with two topics having to do with the potential usefulness of sodium fluoride as a method of treatment of metabolic bone diseases; the strength of fluorotic bone and the mechanism of action of fluoride on bone. Before doing this, we wish to emphasize that, although we will deal with the concept of using sodium fluoride in the management of human osteoporosis, we do not believe its use currently is justified, either as a preventative measure or for therapy of osteoporosis. Although information brought out in this volume is encouraging, we still do not have adequate knowledge of efficacy, safety and how to properly control therapy. Therefore, its use in humans should be limited to appropriately designed clinical studies.

I. STRENGTH OF FLUOROTIC BONE

The practical issue one is faced with in attempting to access the therapeutic effect of sodium fluoride is entirely different than that with any other form of treatment of osteoporosis. This is because, in the case of every other currently used method of treatment, there is a high presumption that bone formed during the period of treatment is of good quality and, therefore, that if an increased bone mass were demonstrated, it could be assumed that bone strength would be increased. The problem is that there has not so far been a convincing demonstration that these other agents cause an increased bone mass. In the case of fluoride, there is no reasonable doubt that bone mass can be increased, but the significance of such change is uncertain, since the histological appearance of fluorotic bone is abnormal and its physical strength could not be assumed to be normal. Therefore, it is critical to establish whether the strength of fluorotic bone is normal, decreased or increased.

It is surprising that, in spite of a number of studies having been carried out (1), no definite conclusions can be made about the strength of bone when doses comparable to what has been given to humans are used. This

* This work was supported in part by grants from the U.S. Public Health Service (AM-9096 and AM-5498).

is because most of the studies were carried out on bone from experimental animals given very high doses of fluoride, while in humans with industrial or environmental exposure, the dose of fluoride was unknown, but very often probably higher than has been used to treat osteoporosis. There is no question that very high doses of fluoride will damage bone, causing severe osteomalacia (2) and osteoporosis (3) The question is whether, at a lower dose such as has been given to humans with osteoporosis, the material strength of bone would still be normal.

Methods. In order to evaluate this question, we have reared Sprague Dawley rats on a nutritionally adequate diet containing 0 6% calcium, 0 4% phosphorus and two units of vitamin D per gram, but no fluorine. In order to reduce the exposure to fluoride during intra-uterine life and nursing, the mothers were placed on the same diet, starting 5 days before delivery. When weaned at 21 days of age, sixty female rats were divided into five groups, which took distilled water containing 0, 5, 15, 30 and 70 ppm fluoride, respectively for 16 weeks. The animals were killed, weighed and the two femora and first four lumbar vertebrae were dissected free. One femur was kept moist at 4°C until testing. It was weighed and marked in the exact center with a pencil. The bone then was placed across a 1 cm bridge, in such a manner that positioning was uniform from bone to bone, and loaded perpendicular to its long axis. The load was applied at the mid-point of the bone at the rate of 5 mm per min until it fractured, using an Instron testing instrument. Cortical and medullary thicknesses at the mid-point of the contralateral femur were measured from enlarged photographs of x-rays Fluoride concentration of the second lumbar vertebra was measured on solutions prepared from alkaline ashed samples, using a fluoride specific electrode Fluoride concentration was expressed in terms of the wet weight of the bone.

The significance of the differences found between control animals and groups given different doses of fluoride was evaluated by means of the t test, using a two tailed distribution Multiple regression analysis was used to evaluate the dependence of a number of parameters on the concentration of fluoride in bone

Results The accuracy of the measurement of bone strength was tested by comparing the stress at failure of both femurs of a separate group of control animals. The results are listed in Table 1, and show a high degree of comparability between the force necessary to fracture the two femurs of any given rat (coefficient of variation was 1.0%) but, even though the rats were comparable as to strain, age, sex, weight and diet, a greater variability from rat to rat (coefficient of variation was 8 6%)

Table 1. Comparison of the stress at time of fracture of the two femurs of control rat

Rat number	*Stress at fracture (kg)*	
	Right	*Left*
1	16.2	16.0
2	21.5	21.4
3	15.7	16.1
4	17.3	16.8

Table 2 presents several measured parameters in the five groups of rats. All animals gained weight at the same rate. The concentration of fluoride in the bones of the control animals was very low and the differences between groups were marked. Bone weight, cortical thickness and bone diameter all were significantly increased in the rats receiving the highest dose of fluoride. Bone strength was significantly increased in the animals given 30 ppm fluoride in the drinking water, as compared to the control group. There was no significant difference in deformation of the bones as they were stressed in any of the groups. When a regression analysis was done to test body weight and bone fluoride concentration together against bone weight as the dependent variable, each was found to contribute significantly to the strength of the regression. Thus, this analysis confirmed a significant overall positive correlation between the concentration of fluoride in bone and bone weight ($F = 12.4$, $p < .01$) which was independent of and in addition to the expected high degree of correlation between bone weight and body weight ($F = 39.8$, $p < .0001$).

The relationship between bone strength and fluoride concentration in bone is illustrated in Figure 1. As also indicated by the data in Table 2, stress at fracture was similar in control animals and those having more than 4,000 ppm fluoride in bone (which were those receiving the highest dose of fluoride). However, bones from animals in the middle range (2,000 to 4,000 ppm fluoride in bone; the group given 30 ppm fluoride in water) withstood more stress than animals either on a low or high dose. The relationship between stress at fracture and several other parameters was examined, using multiple regression analysis. When all of the animals were tested, no significant correlation was found between bone strength and any of the following; body weight, bone weight, bone length, bone diameter, cortical thickness or fluoride concentration. However, when the animals receiving the highest dose of fluoride (70 ppm) were excluded from the analysis, a significant correlation was found between bone strength and fluoride concentration ($F = 5.9$, $p < .01$). The strength of the regression was not significantly

Table 2 Measurements made on rats exposed to different doses of fluoride in drinking water for 16 weeks

Parameter measured	*units*	*Concentration of fluoride ion in water (ppm)* 0	5	15	30	70
Number of rats per group		*11*	*7*	*9*	*6*	*8*
Body weight	gm	265 ± 26†	264 ± 15	285 ± 25	295 ± 25	274 ± 35
Femur weight	mg	795 ± 66	788 ± 61	839 ± 61	871 ± 72	868 ± 86**
Femur cortical thickness	mm	1 40 ± 01	1 39 ± 01	1.39 ± .01	1.44 ± 01	1.48 ± .01*
Femur total diameter	mm	3.28 ± 19	3.30 ± 07	3.43 ± 17	3.43 ± .19	3.55 ± .17**
Femur stress at fracture	kg	13 1 ± 1 5	13 5 ± 2 5	12 6 ± 2.1	16.5 ± 1.9**	14 2 ± 2 7
Femur strain at fracture	mm	1.13 ± .43	0 97 ± 08	1 27 ± .23	1.20 ± .20	1.49 ± .23
Vertebral weight (average of L 1–4)	gm	653 ± 82	676 ± 75	665 ± 65	737 ± 100	727 ± 111
Vertebral fluoride concentration (L 2)	ppm	42 ± 8***	412 ± 81	1380 ± 223	2980 ± 486	5820 ± 620

† Mean ± standard deviation
* *Significantly different from control group* ($p < .05$).
** Significantly different from control group ($p < .01$)
*** All groups significantly different from each other.

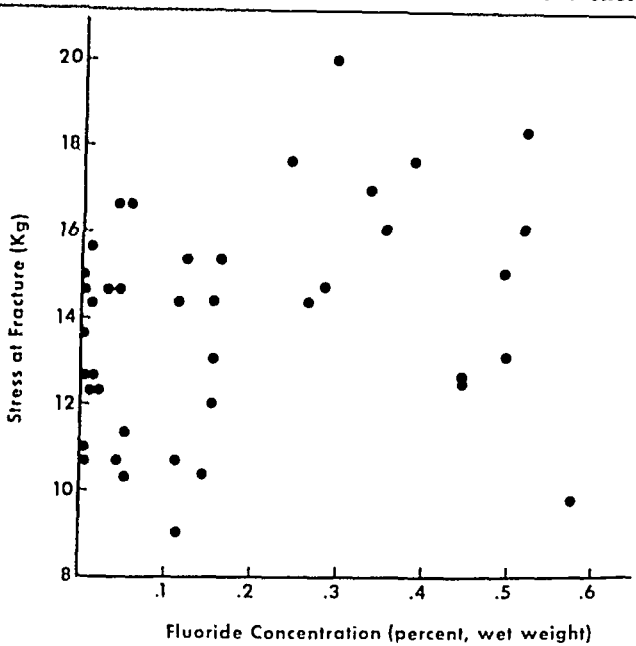


Fig. 1. The stress at fracture of the femur is plotted on the ordinate and fluoride concentration in the entire 2nd lumbar vertebra of the same animal on the abscissa.

increased when the following were added to the regression; body weight, bone weight, bone length, bone diameter and cortical thickness. Thus, there was a significant positive correlation between fluoride concentration and bone strength which was not contributed to by these other factors.

Comments. This study was designed to provide a range of doses from zero to a mildly toxic level. The precautions taken to ensure low concentration in bone of the control rats are necessary because of the high concentration of fluoride normally found in rat chow. Our success in reducing exposure is shown by the low concentration of fluoride in bone of the control group; only $^1/_{10}$ of that in the animals given 5 ppm fluoride in their drinking water (see Table 2). We chose 70 ppm fluoride for our highest dose because previous studies have shown that animals given more than this amount gained weight less rapidly, presumably as a result of intestinal or other soft tissue toxicity.

Although we have no measurements of the absolute dose given these rats, some assessment of it can be based upon measurements of body weight and water intake. Our best estimation is that the rats given 15 ppm took 1.2 to 1 mg fluoride ion/kg body weight/day, which is comparable to the dose range used in humans, while the rats given 70 ppm fluoride received about 4 times as much.

Because the femur not used for testing was saved for another study (4), the concentration of fluoride was measured *in the 4th lumbar vertebrae* rather than the femur. We assume that the concentration of fluoride in the two bones would be different but related *in some regular way, so that what* was found in the vertebrae can adequately characterize the state of fluorosis of the femur. The concentrations obtained in the control group were lower than found except from animals reared under similarly controlled conditions. The concentrations of fluoride in bone of animals given 5, 15 and 30 ppm fluoride covered the range observed in human bone where there has been no unusual exposure, with the concentration of fluoride of rats receiving 5 ppm fluoride being similar to what is found in childhood and the bone from rats given 30 ppm fluoride being comparable to what is found in bones of old people and in some cases of minimal fluoride induced sclerosis (1, 18). The rats receiving the highest dose had concentrations of fluoride in bone similar to what is found in mild osteosclerosis (1, 18).

There is no question that what was being tested in this study was uniformly fluorotic bone, as the skeleton present at the time of weaning is entirely replaced by new bone well before 16 weeks of age. Thus, the observed mechanical properties could not have been due to residual, non-fluorotic bone

The relationship we observed between bone mass and fluoride dose appears rigorously established Not only was there a significant difference between groups (Table 1) but there was also a significant positive overall correlation between bone mass and fluoride concentration which was not dependent upon the relationship between bone mass and body weight. This indicates that, under the conditions of this study, fluoride had the expected biological effect Measurements on parameters of bone growth and resorption in these same animals are reported elsewhere (4)

In view of opinions that fluorotic bone is brittle, it was of interest to find no evidence of any difference in strain (deformity of the bone as it was stressed to the point of fracture) in any of the groups (Table 1).

The basic objective of this work was to determine whether or not the strength of fluorotic bone is normal If so, or if it were increased, then it would be permissible to assume that the demonstration of increased bone mass in a fluoride treated patient could indicate that he was being aided by therapy (We define bone strength operationally as resistance to fracture under the conditions we used to apply stress).

All of the findings presented support the conclusion that, within the range of doses used and under the specific conditions of this experiment, there was no reduction of bone strength. Indeed, we found significantly increased

resistance to fracture in the animals given 30 ppm fluoride in drinking water, indicating increased bone strength in this group. However, the conclusion that bone strength was increased must be regarded as preliminary, requiring verification by similar findings in another comparable experiment. This is because the specific hypothesis we set out to test was that, within the dose range examined, there would be a linear relationship between dose and bone mass and strength. This was found in the case of bone mass and the conclusion in respect to bone mass is valid. However, the relationship found between dose and bone strength is non-linear (Fig. 1) and, therefore it is possible that the calculated statistical significance is spurious. However, the relationships observed remain as the most probable correct description and as a basis for future confirmatory experiments. These results indicate that bone strength in animals receiving 0, 5, 15 and 30 ppm fluoride probably was positively correlated with the concentration of fluoride in bone, but not with bone weight or body weight. They indicate no correlation with bone diameter or cortical thickness, but these latter results cannot be viewed with the same confidence as the correlations involving bone strength, bone mass and body weight, since the measurements of cortical width and bone diameter were made on the contralateral femur rather than the one that was fractured. Nevertheless, they probably are valid, since the strength of the two femurs of any given rat is almost identical (Table 1, coefficient of variation 1 %). The reason for the differences in bone strength, both among the control and fluoride treated rats, is not clear. It could be related to factors such as tensile strength, elasticity and viscosity, that contribute to the material strength of bone, or to differences in gross architectural features such as bone diameter, cortical thickness and shape or location and size of intra-cortical vascular spaces. We would expect that parameters of mass, such as weight and cortical thickness, and that bone diameter (4) would be important in determining the resistance of the femur to stress applied perpendicular to its long axis at the center of the shaft. If the observations reported here, that they were not, were to be confirmed in another group of rats, it would raise the possibility that the material strength of bone had been increased as a result of fluoride exposure.

II. MECHANISM OF ACTION

Although so little is known at this time that any consideration of mechanism of action of fluoride on bone must necessarily be highly speculative, we believe that we must analyze what we do know, so as to have a framework within which a rational approach to therapy can be constructed. Accordingly,

we will discuss in some detail what we propose may be the mechanism of action of sodium fluoride in bone; but emphasize first that much of what we will say is speculative (but no more so than would be necessary for presentation of any other concept of fluoride action on bone).

The most widely accepted concepts of how fluoride might act on bone (1, 5–7) include one or more of the following: a) Concentration of fluoride in bone, with formation of mixed hydroxyfluorapatite, presumably mainly in bone laid down during the time of fluoride administration. b) Because of decreased crystal solubility, inhibition of osteoclastic bone resorption; particularly resorption of bone laid down during the period of fluoride administration c) Secondary hyperparathyroidism, presumably to maintain calcium homeostasis, leading to d) increased differentiation of osteoclasts* and osteoblasts.

There is a substantial weight of evidence for several of these concepts. There is no doubt at all that fluoride is highly concentrated in bone. Evidence for reduced solubility includes the demonstrations that fluorotic bone is less soluble (8) and evidence that a mixed hydroxyfluorapatite is formed after either long term exposure (9) or as a result of a therapeutic trial lasting about one year (10) Bone of animals treated with sodium fluoride has been found to show a lower resorptive response when large exogenous doses of parathyroid hormone are given (11). Furthermore, there is direct and indirect evidence for secondary hyperparathyroidism in fluoride treated animals and man Histological appearance (2) and biochemical changes (7) have been found which are suggestive of hyperparathyroidism, and structural and chemical evidence of parathyroid hypertrophy has been found in fluoride treated humans (12) and rabbits (13), although not in rats (14) Most compelling is the direct measurement of the concentration of parathyroid hormone in blood, using the radioimmunological assay method. A five fold increase has been found when sheep were treated with 100 mg fluoride ion per day (13).

Although all of these points all must be accounted for in discussing the mechanism of fluoride effect, there are several facts that are difficult to explain in terms of the concept stated above. The onset of fluoride effect in

bone is far too rapid to be related in any significant way to the bone laid down during the period of fluoride administration. This is obvious when one considers the rate of renewal in the adult skeleton; it takes 10 to 20 years or more for half of the mass of human bone to be replaced. In contrast, the effect of fluoride on bone starts to be expressed within weeks to a month or so after treatment is started. YATES, DOTY and TALMAGE (15) found evidence of hyperparathyroidism within days of starting to treat rats with sodium fluoride and FACCINI and CARE (13) found the concentration of parathyroid hormone in blood of sheep elevated one week after starting treatment. Calcium retention (12, 16) and increased osteoblastic differentiation (17) have been found within 2 to 3 months of starting to treat humans with sodium fluoride. These periods all are so short that it appears very unlikely that a significant part of the crystals of bone would have been formed as hydroxyfluorapatite. Thus, although the reduced solubility of fluoride laden bone crystals in bone laid down during fluoride administration unquestionably would play a significant role under conditions of prolonged administration, it is doubtful that it is necessary or important in the *initiation* of fluoride effect. Furthermore, reduced crystal solubility probably is not a major factor in *maintaining* effect of fluoride on bone. If it were, one would expect to see the effect of fluoride continue after treatment had been stopped, until much of the fluoride had been excreted from the skeleton. The rate of excretion of fluoride from the skeleton has been determined and is very slow; about 1% of the dose remaining per week (18). Although additional evidence on the point is needed, there is general agreement that the bone disease does not progress once exposure to fluoride is stopped. We and others have found that calcium retention ceased several months after treatment was discontinued; before any significant fraction of the fluoride deposited in the skeleton during the previous treatment (32 months in our patient) had been excreted (12, 19). Furthermore, the data presented by SCHENK et al. (17) (p. 53) can be interpreted as showing a strong relationship between dose and almost all of the parameters measured, including the rate matrix formation. In the first year, the dose usually was high (80 mg fluoride per day or more) and the rate of matrix formation and several other parameters were elevated, while the histological appearance of the matrix and its mineralization both were grossly abnormal. In the second year of treatment, when much smaller doses usually were given (50 mg fluoride per day or less), the rate of bone formation and several other parameters still were elevated, but less so, and the histological appearance and mineralization of the matrix both appeared normal. These points all support a conclusion that the action of fluoride on bone may cease much sooner after therapy is stopped than would be expected

if the action were proportional to some effect of fluoride in bone formed during the period of exposure.

For the reasons cited above, we believe one must look to some other property of fluoride than its effect on crystals in newly formed bone. The hypothesis we favor follows on our earlier speculations about the action of fluoride in bone (16) and is based principally upon the two most conspicuous aspects of fluoride metabolism in mammals; that it is concentrated at the surfaces of bone and that it is toxic to cells.

The first assumption is that fluoride is not distributed evenly throughout bone, but rather, that it is highly concentrated in two specific regions. These are, a) those areas of bone formed during the time of administration, when the blood concentration of fluoride is high and, b) the surface layer of bone immediately bordering upon the osteocyte lacunae and canniculae The former localization is obvious and well accepted, but the latter has not been suggested and deserves comment. In principal, during administration of fluoride, its concentration in blood and extracellular fluid rises, somewhat proportionately to the dose. The extracellular fluid perfuses the osteocyte lacunae and canniculae, bringing fluoride into contact with these surfaces of bone. Presumably, it both is adsorbed at high concentration at this interphase and incorporated into the crystal lattice, so that the concentration of fluoride in this region may conceivably be quite high. Since it must pass from this region if it is to reach interlacunar bone, it can be concluded that its concentration in this region will be greater than in interlacunar bone. However, it is likely that fluoride ion would not migrate deeply into interlacunar bone, since crystals in fully calcified bone are so closely packed as to partially exclude fluid and strongly impede diffusion of ions. Accordingly, we propose that fluoride would be concentrated at the lacunar and canniculer surfaces, rather than evenly distributed throughout interlacunar bone, as illustrated schematically by Fig. 2. We have not been able to evaluate this possibility directly because of analytical difficulties and, therefore, offer as an analogy, what is found when one gives tetracycline; a molecule which also binds firmly to bone mineral and which can be distinguished in bone because of its fluorescence. Within the first day or so of its intravenous administration, tetracycline is found in areas of bone apposition, as expected, but also in a sharp zone along the borders of many of the osteocyte lacunae and canniculae It is this distribution which accounts for the fluorescence in areas of preformed bone after a dose of tetracycline is given; the interlacunar bone remaining unlabeled. The tetracycline fluorescence in this zone is not permanent but, rather, if additional doses are not given, diminishes gradually over a period of weeks (20) We interpret this tetracycline uptake to be

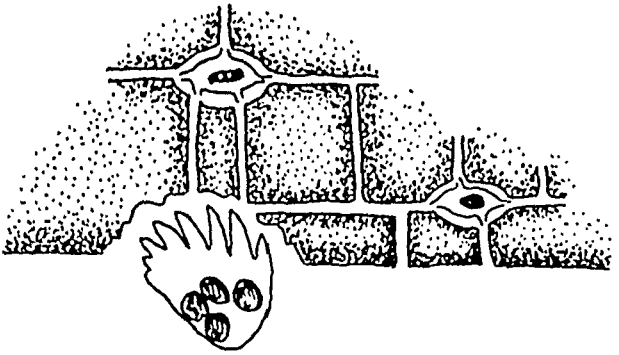


Fig. 2. A schematic representation of the distribution of fluoride in bone formed prior to the time treatment with fluoride was started. The regions of intense stippling represent areas of high local concentration of fluoride.

due either to deposition at crystal surfaces in areas of low mineral density, or to binding with newly precipitated crystals in this region, or both. We interpret the subsequent gradual reduction in fluorescence as the result of mobilization of some of the mineral from the border of osteocyte lacunae and canniculae, presumably as a result of osteocyte metabolic activity. The calcium, phosphate and tetracycline so mobilized presumably pass either back into the blood, or deposit elsewhere in the cannicular system. The assumptions we make are that tetracycline is a fair marker for such distribution of calcium and phosphate and that fluoride in bone would have the same distribution as tetracycline.

On the basis of the evidence and opinions discussed above; we conclude a) that fluoride in preformed bone is concentrated mainly in a surface located layer of mineral at the border of osteocyte lacunae and canniculae and b) that fluoride in extracellular fluid of bone is in a rather slow equilibrium with the fluoride in this mineral phase, as a result of osteocyte regulated removal and deposition of mineral in this region. If these conclusions are valid, one can derive the following. Since the amount of fluoride which would remain in solution in extracellular fluid adjacent to apatite of bone presumably is very low, bone cells would not be exposed to high concentrations when they were not resorbing bone. However, it is evident that any cells which resorb bone necessarily would be thereby exposed to a significant concentration of fluoride. This specific exposure would hold for osteocytes and osteoclasts throughout bone, both of which would be subjected to a concentration of fluoride which would be approximately proportionate to the intensity of the resorptive process. It could be speculated that the exposure of osteocytes, which are entirely surrounded by a surface on which fluoride probably is

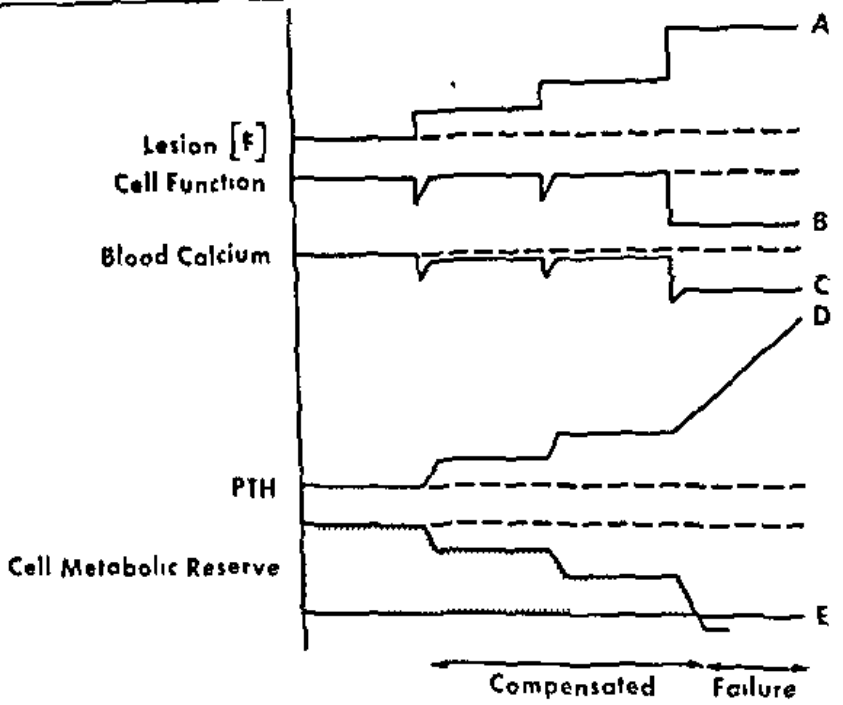


Fig 3 The severity of the homeostatic lesion is plotted as a set of discrete increments (A). This lesion is assumed to be inhibition metabolic function of osteoclasts and osteocytes resulting from each increase in fluoride in adjacent bone Each decrement of resorption efficiency (B) is seen as causing hypocalcemia (C) This in turn causes an increased secretion of parathyroid hormone (D), to just that degree necessary to drive osteocytic and osteoclastic resorptive processes back to the original level, thereby, restoring eucalcemia However, each parathyroid hormone

centrations or activities

concentrated and which exist further away from the blood stream, would be subjected to a higher concentration of fluoride upon resorbing bone than would be the osteoclasts

What fluoride does to these cells is unknown.* We believe the action on bone cells would be a toxic one and that the consequence of a high regional concentration of fluoride would be inhibition of the resorptive function.

* An interesting possibility is that it might initiate the same sequence of events as does parathyroid hormone, through directly activating 3′ 5′ cyclic AMP. Direct activation *in vitro* requires 200 ppm fluoride, a concentration which conceivably could occur in the extracellular fluid of bone if resorption occurred at a region of high surface concentration, but which is very much above what would be consistent with cell function. A lower concentration, which could be tolerated by living cells, might be effective *in vivo*. However, the fact that parathyroid hormone concentration rises when fluoride is given is against this possibility.

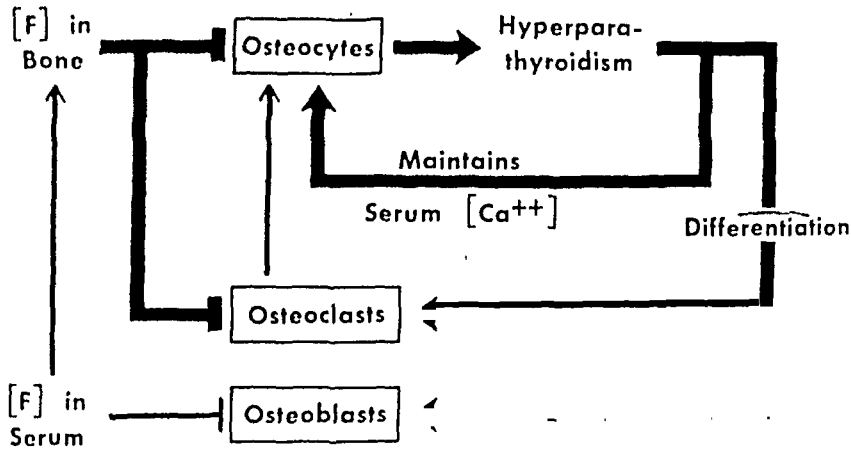


Fig. 4. A schematic representation of the effects of fluoride on calcium homeostasis and bone metabolism. The lines ending in a perpendicular bar indicate inhibition; those ending in an arrow indicate stimulation.

There is ample evidence that fluoride passes into cells and that it inhibits numerous enzyme activities (1, 21) and therefore, the concept that metabolic function might be inhibited when cells are exposed to high fluoride concentrations is reasonable. The high local concentration of fluoride presumably would promote formation of hydroxyapatite and reduced crystal solubility in these regions, further inhibiting resorptive efficiency. The probable consequence would be an increase in parathyroid hormone secretion to just that degree necessary to compensate for the inhibition of homeostatic bone resorption; thereby maintaining the concentration of calcium in blood at or near the normal level. An analysis of how these events would follow upon a fluoride induced lesion of bone resorption is presented in Fig. 3.

The overall metabolic consequences of fluoride incorporation in bone are illustrated in Fig. 4. In this figure, it is assumed for convenience, that osteocytic resorption has mainly to do with calcium homeostasis and that osteoclastic resorption has mainly to do with bone remodeling. The concept presented is that the degree of hyperparathyroidism exactly compensates for the fluoride induced inhibition of homeostatic bone resorption. I assume for the purpose of this discussion that parathyroid hormone stimulates osteoblastic as well as osteoclastic differentiation and therefore, that there is a stimulus to osteoblastic differentiation that is proportional to the severity of the inhibition of homeostatic resorption (ultimately, to concentration of fluoride in blood). There is a great deal of indirect evidence to support the concept that parathyroid hormone directly stimulates osteoblastic differentiation, but it remains unproven, Although some direct action of fluoride on

osteoblastic differentiation cannot be excluded and certainly may occur, it seems less likely than an indirect action based upon secondary hyperparathyroidism, as proposed here.

Assuming that there is increased osteoclastic and osteoblastic differentiation, the expression of the former would be somewhat offset by the cytotoxic action and the reduction of crystal solubility caused by fluoride However, the same factors would not operate to the same degree to inhibit the expression of osteoblastic metabolic function. Accordingly, an increased rate of bone formation with a slightly increased, normal, or even decreased rate of osteoclastic bone resorption would be predicted as the overall result

Clinical implications. Fig. 5 presents a therapeutic rationale in the form of an analysis of the effects of administration of fluoride on bone formation and resorption, based on the assumptions and concept already discussed. If this is valid, all of the effects of fluoride on bone follow in a single sequence; that is, the toxic effects can be seen as exaggerations of the potentially therapeutic actions. Furthermore, the implications are that the concentration of fluoride in blood may be the critical determining factor, both for a potentially therapeutic effect and of the toxic effects; a point that recently has been given deserved emphasis by TAVES (22). It is important, therefore, to establish as soon as possible, the relationships between dose, period of administration, concentration in bone and concentration in blood Given this and some idea of the lower limit of toxicity, a rational approach to-

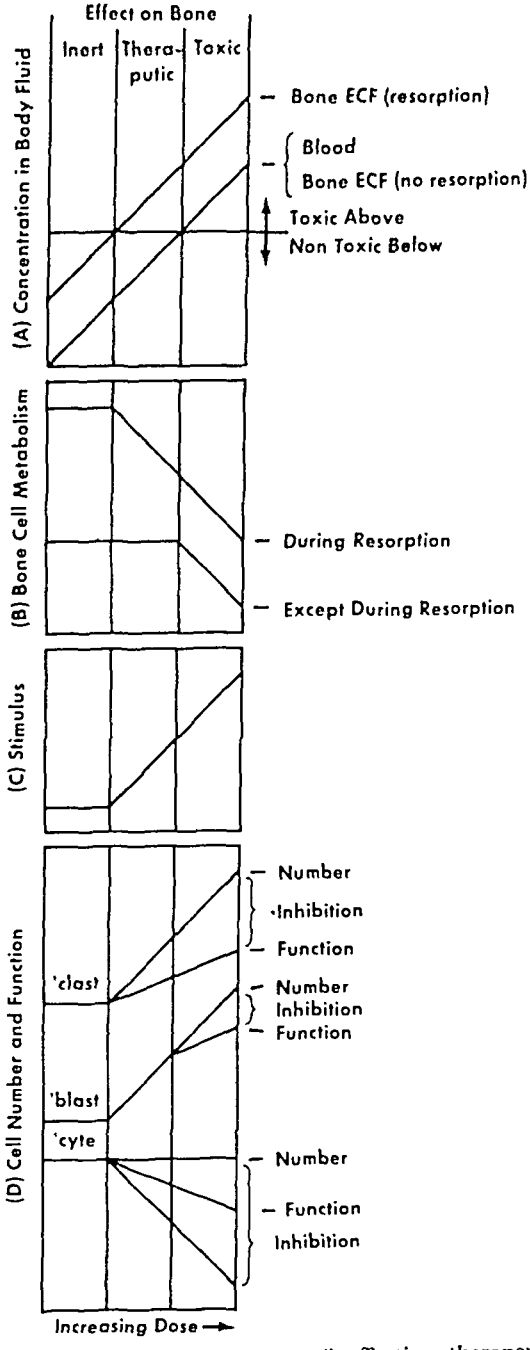


Fig. 5. The abscissa represents three different dose rates (ineffective, therapeutic and toxic). The lower line in Fig. 5A shows the concentration in blood and extra-

cellular fluid (ECF) With high dose rates, the concentration raises to the point where it is toxic to cells. The upper line shows the increased concentration of fluoride in ECF in the region of cells that are actively resorbing fluoride laden bone. Fig. 5B shows the resulting inhibition of bone cell function. Osteoblasts and, except during active resorption, osteoclasts and osteocytes are inhibited only at very high dose rates, as shown by the lower line The upper line indicates the inhibition of osteoclasts and osteocytes as a result of a high local concentration of fluoride produced by resorption of fluoride laden bone. Fig 5C illustrates the hypotheticated stimulus to bone cell differentiation, given as a function of inhibition of resorption In the absence of direct evidence that points elsewhere, this mechanism is presumed to by hyperparathyroidism and the line in 5C is presumed to *represent the concentration* of hormone in blood. Fig 5D illustrates the consequences upon the number of cells and function of these cells in bone. Osteoclastic differentiation results in an increased number of cells but, *because of fluoride* exposure and reduced crystal solubility, their potential function is inhibited, proportionate to the concentration of fluoride in bone at the surfaces undergoing resorption. Osteoblasts also are differentiated but function *is inhibited only* when *blood levels are high.*

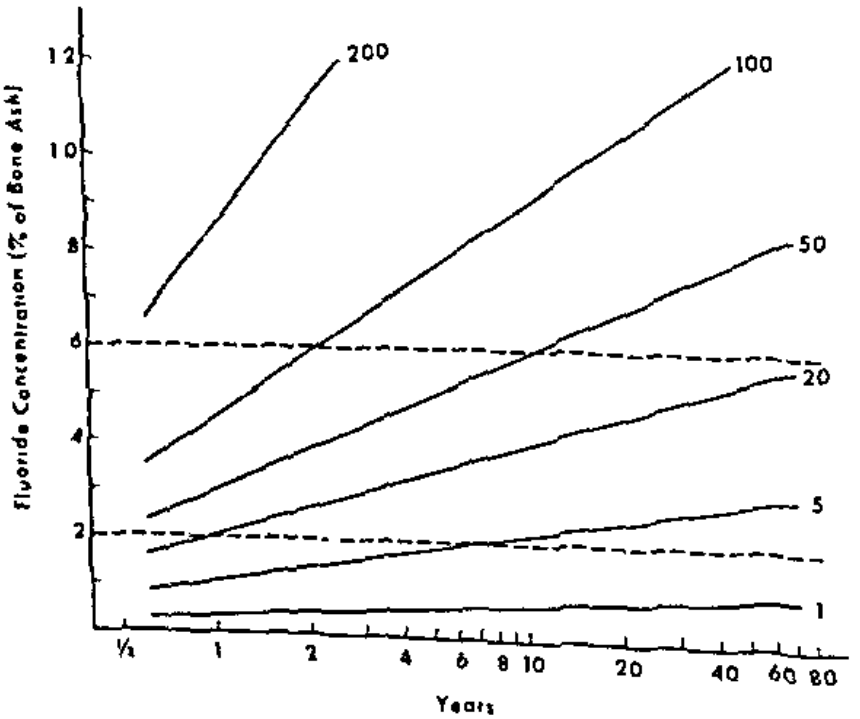


Fig 6 The relationship bet
fluoride in bone, e
humans and lower
and 200 mg of the fluoride ion per day. The dashed lines enclose a range of concentrations of fluoride in bone suggested in the text as potentially therapeutic

normal appearing bone and no evidence of generalized toxicity when the concentration in blood is maintained between 0.1 and 0.2 ppm, and that concentrations above this are associated with increasingly severe histological abnormalities in bone and generalized manifestations of toxicity (22). Using the small amount of data which is available, TAVES concludes that administration of around 60 mg fluoride ion per day probably will maintain the blood concentrations in the range between 0.1 and 0.2 ppm, but that there are considerable individual differences, depending upon different rates of absorption and of renal and bone clearances. Thus, all of the methods used above to evaluate the amount of fluoride which might be most likely to stimulate bone formation with minimal adverse consequences point towards a dose in the neighborhood of 50 mg of the ion per day, possibly reduced after a year or so.

REFERENCES

1 HODGES, H. C., and SMITH, F. A.: Fluorine chemistry, vol. IV (J. H. SIMMONS, ed.). Academic Press, New York (1965).
2 BAYLINK, D. J., and BERNSTEIN, D. S.: The effects of fluoride therapy in metabolic bone disease. Clin. Orthop. *55*, 51 (1967).
3 COMAR, C. L., VISEK, W. J., LOTZ, W. E., and RUST, J. M.: Effect of fluoride on calcium metabolism and bone growth in pigs. Amer. J. Anat. *92*, 361 (1953).
4 BAYLINK, D., WERGEDAL, J., STAUFFER, M., and RICH, C.: Effects of fluoride on bone formation, mineralization, and resorption in the rat, in *Fluorine in Medicine* (T. L. VISCHER, ed.). H. Huber, Berne (1970).
5 JOHNSON, L. C.: Histogenesis and mechanisms in the development of osteofluorosis, in *Fluorine chemistry* (J. H. SIMMONS, ed.), vol. IV (H. C. HODGE and F. A. SMITH). Academic Press, New York (1965), p. 424.
6 FACCINI, J. M.: Fluoride and bone. Calc. Tiss. Res. *3*, 1 (1969).
7 NICHOLS, G. jr., FLANAGAN, B., and WOODS, J. F., in *The parathyroid glands: ultrastructure, secretion and function* (P. J. GAILLARD and R. V. TALMAGE, eds.). Univ. Chicago Press, Chicago (1965), p. 243.
8 MCCANN, H. G., and BULLOCK, F. A.: The effect of fluoride ingestion on the composition and solubility of mineralized tissues of the rat. J. Dent. Res. *36*, 391 (1957).
9 POSNER, A. S., EANES, E. D., HARPER, R. A., and ZIPKIN, I.: Xray diffraction analysis of fluoride on human bone apatite. Arch. Oral. Biol. *8*, 549 (1963).
10 GRON, P., MCCANN, H. G., and BERNSTEIN, D.: Effect of fluoride on human osteoporotic bone mineral, J. Bone Joint Surg. *48-A*, 892 (1966).
11 HAVIVI, E., and GUGGENHEIM, K.: The effect of copper and fluorine on the bone of mice treated with parathyroid hormone. J. Endocrinol. *36*, 357 (1966).
12 BERNSTEIN, D. S., and COHEN, P.: Use of sodium fluoride in the treatment of osteoporosis. J. Clin. Endocrinol. *27*, 197 (1967).
13 FACCINI, J. M., and CARE, A. D.: The effect of sodium fluoride on the ultrastructure of the parathyroid glands in sheep. Nature (London) *207*, 1399 (1965).

14 Raisz, L. G., and Taves, D. R.: The effect of fluoride on parathyroid function and responsiveness in the rat. Calc. Tiss. Res. *1*, 219 (1967).
15 Yates, C., Doty, S , and Talmage, R V.: Effects of sodium fluoride on calcium homeostasis Proc. Soc. Expt. Biol. (N.Y.) *115*, 1103 (1964).
16 Rich, C., Ensinck, J , and Ivanovich, P.: The effects of sodium fluoride on calcium metabolism of subjects with metabolic bone diseases. J. Clin. Invest. *43*, 545 (1964).
17 Schenk, R. K., Merz, W. A., and Reutter, F. W.: Fluoride in osteoporosis, in *Fluoride in medicine* (T. L. Vischer, ed). H. Huber, Berne (1970).
18 Largent, E. J.: *Fluorosis*, Ohio State Univ. Press, Columbia, Ohio (1961).
19 Rich, C., and Ivanovich, P : Response to sodium fluoride in severe primary osteoporosis Ann Int. Med. *63*, 1069 (1965)

21 Cimasoni, C Fluoride and enzymes, in *Fluoride in medicine* (T. L. Vischer, ed) H Huber, Berne (1970)
22 Taves, D R A new approach to the treatment of bone diseases with fluoride Fed Proc (in press; 1969).
23 Taves, D R Normal human serum fluoride concentrations. Nature *219*, 192 (1966)

Mechanism of action of fluoride in reducing dental caries

G. N. JENKINS

Before describing current views on the mode of action of fluoride in reducing dental caries it seems desirable to summarise the main clinical observations on its caries-reducing action.

1. Throughout the world it has been found that water-borne fluoride reduces dental caries in children. The optimum dose produces about 50% reduction and varies from 0.7 ppm (in warm climates with consequently a high water content) to about 1.5 ppm (in cooler climates). Higher doses do not have a greater effect in reducing caries and above 2 ppm mottling becomes objectionable. Artificial fluoridation has the same effect as fluoride present naturally.

2. Owing to the known difficulties in scoring caries in adults there is less accurate information on the caries-reducing effect of a life-long intake of fluoride on older people. The limited evidence which has been collected suggests that the effect is present throughout life but may diminish somewhat in older age groups.

3. For the full effect it is necessary to receive the fluoride during enamel formation (i.e. the first eight years of life) and afterwards. Some benefit (about one third of the maximum) is derived if fluoride is first received after the teeth are fully formed.

4. The application of high concentrations of fluoride as dentifrices (usually 0.1% F), mouth washes (0.025% daily or 0.1% F fortnightly) and painting on to the tooth ("topical application") once or twice yearly (usually of 1% F in various forms) are also clinically effective. It cannot be assumed that these very high concentrations work in the same way as 1 ppm in the water.

5. The effect of fluoride on caries is far greater than that of any other substance or procedure except perhaps the total elimination of sweet-eating between meals.

Several quite different mechanisms have been suggested for the action of fluoride in caries and current evidence suggests that all may play a part but their relative importance is uncertain.

EFFECT OF SOLUBILITY OF ENAMEL CRYSTALS

Hydroxyapatite, the main constituent of bones and teeth, has a great affinity for fluoride which exchanges with the hydroxyl ion to form fluorapatite which has usually been regarded as a less soluble crystal. It is known that most of the fluoride in the body is concentrated by the calcified tissues, especially where they have access to body fluids, e.g. the endosteal surface of bone and the outer surface of enamel. When teeth or powdered enamel are shaken with solutions of fluorides in vitro it can readily be shown that the introduction of fluoride reduces the solubility-rate of the tooth in acids. This simple experiment has such dramatic results that many have assumed that reduction in solubility explains the clinical effect in caries. There have been four attempts to test the theory by comparing the solubilities of teeth from people living with or without fluoride in their water (8). All have given general support to the theory because the teeth from the fluoride areas were found on the average to be less soluble than the controls, but the effect is small and not always statistically significant. Although it is a reasonable assumption that reductions in enamel solubility do reduce caries, it has never been proved.

The largest effect on solubility has been found in enamel which is already attacked by early caries. In early lesions, the enamel becomes permeable and fluoride can enter more readily: also, a low pH is known to favour fluoride uptake by apatite, and the pH in carious areas is lower than that of the mouth as a whole Enamel already showing carious changes may be 20% less soluble than intact enamel in the same teeth (4).

THE MEANS BY WHICH FLUORIDE REDUCES SOLUBILITY

Recent thinking, while accepting that fluoride may reduce the solubility of enamel, has raised doubts as to whether this can be explained on the basis that hydroxyapatite is converted into fluorapatite by contact with fluoride From recent results it is not certain that fluorapatite really is less soluble than hydroxyapatite (6), but the question has not yet been settled

Other mechanisms of affecting solubility have been suggested and they are worth discussing in some details because these points may also be important in the relation between fluoride and bone

Alternative suggestions are that fluoride increases the size of apatite crystals and reduces the number of crystal defects–both these effects are known to occur in bone under the influence of fluoride (12), but whether this applies

to enamel is less certain (5). Another related suggestion is that fluoride competes with carbonate during apatite formation and there are reasons for believing that a high carbonate concentration in enamel lowers its resistance to caries. Some evidence supports this theory but it has been challenged.

Another difficulty in the solubility theory is that many ions are known to reduce the solubility of enamel in vitro (e.g. zinc, copper, iron and cadmium), but none of these have been shown to influence caries. Fluoride seems unique in its caries-reducing effect but not unique in its effect on solubility. A suggested solution of this difficulty is that fluoride reduces solubility in some unique manner and one reaction has been discovered which may throw light on this possibility. If phosphoric acid is titrated against lime water until a precipitate of calcium phosphate forms, and the mixture is allowed to stand for some days in the presence of fluoride, the pH of the supernatant and the composition of the precipitate change (1). One interpretation is that the first precipitate to form is $CaHPO_4$ which, in the presence of fluoride, gradually changes into apatite. It is thought that apatite is the most stable and least soluble crystal which calcium phosphate can form. This theory suggests that the action of fluoride in caries is its unique ability to encourage the formation of this substance rather than other less stable crystal forms. This reaction is unlikely to be important during enamel formation because, even in the absence of fluoride in the water, the chief crystal of enamel is apatite and therefore the theory leaves unexplained the reported lower solubility of intact enamel in areas high in water-borne fluoride. It could occur, however, in a caries lesion, which is now thought to go through phases of softening and decalcification alternating with rehardening by reprecipitation of some of the apatite dissolved. It seems likely that fluoride is working on solubility in two ways: (a) modifying the enamel during formation so that it is less soluble (? higher proportion of fluorapatite; ? larger, more perfect crystals), (b) encouraging reformation of crystals in the rehardening phase of caries and increasing the tendency for the crystals to be in the most stable and least soluble form (apatite). The final answer is still uncertain.

MORPHOLOGICAL EFFECTS

Animal experiments have shown that fluoride ingestion during tooth formation leads to smaller teeth with shallower and more rounded fissures (10, 11) and similar observations have been made on human teeth in Hastings, New Zealand (2), although WALLENIUS (14) found that 1 ppm of fluoride was associated with a 1.7% increase in the size of the teeth.

These differences would be expected to lead to less food stagnation, and greater access of saliva which is both alkaline and highly buffered when vigorously stimulated. The effects are very small, however, and it is impossible to decide whether these observed differences would have a significant effect on caries.

ANTI-ENZYMIC EFFECTS OF FLUORIDE

The well-known effect of fluoride as an enzyme inhibitor raises the question of whether this action is exerted on the oral bacteria responsible for caries It has been known for years that many oral bacteria are sensitive to fluoride but the concentrations needed for marked inhibition (say, 10 ppm) seemed much higher than the concentration present in the mouth Saliva and plasma were thought to contain about 0.1 ppmF but recent analytical refinements suggest that the concentration is lower, about 0.02 ppm (7). The situation was transformed when it was discovered that the dental plaque (the layer of modified salivary protein and bacteria on the tooth surface) contained surprisingly high concentrations which were affected by the fluoride of the drinking water. DAWES, JENKINS, HARDWICK and LEACH (3) reported an average of 25 ppm in plaques from children in a "low fluoride town" and 47 ppm where the water contained 2 ppm fluoride, although the range of variation was very large. These concentrations were adequate to inhibit but it was clear that most of this fluoride must be bound in some way, otherwise it would not accumulate The question now became: Is this fluoride bound in a form in which it can inhibit bacterial enzymes? Recent work supports strongly the conclusion that much of the fluoride is contained within the bacteria in a form which does reduce acid production The evidence is that plaques from a "high fluoride town" produced less acid when standing with sugar than comparable plaques from a "low fluoride town (9)

fluoridation [illegible] bacteria have been cultured on media containing varying concentrations of fluoride and after thorough washing the bacteria have been found to contain high concentrations of fluoride and to show inhibition of acid production proportional to their fluoride concentration (9).

In vitro experiments have shown that the synthesis by oral bacteria of intracellular polysaccharides from glucose is sensitive to fluoride (13, 15). These stores of polysaccharide may be of importance in prolonging acid production in the plaque beyond the time when sugar is available during

eating. If this is so, then one action of fluoride might be to reduce the storage and therefore the duration of the fall in pH.

The high concentration of fluoride in plaque raises the question of whether it is wise to remove it by tooth-brushing. The answer seems to be that in spite of its fluoride content the overall effect of plaque is to damage the tooth and promote inflammation of the gingivae and its removal seems desirable.

The source of plaque fluoride is probably saliva. The alternative possibilities are the enamel surface (known to be very high in fluoride, e.g. 1000 ppm) or food and drinks. If fluoride were constantly diffusing from enamel into plaque it would be expected that the fluoride on the outer surface would gradually diminish with age, but most data suggest that it rises. Also, it is known that the fluoride present in apatite is tightly bound and most unlikely to be released. The fluoride concentration of food is low and, even after chewing and mixing with saliva, unpublished experiments in the author's laboratory have shown that the fluoride concentration in the bolus rarely exceeds 0.5 ppm and is usually much lower. Although certain drinks (tea and beer) contain higher concentrations, their contact with plaque is slight and of short duration. Present evidence suggests that the slow but continuous flow of saliva over the plaque is the most likely source of its fluoride.

CONCLUSION

The evidence presented suggests that fluoride probably reduces caries because it possesses a unique combination of properties all of which may play some part. The question to be asked seems not to be: which theory is right? but: what is the relative importance of the various effects which fluoride seems able to exert? As mentioned previously, fluoride must be ingested during enamel formation for its full effect but approximately one third of its total action occurs if fluoride is received later. This implies that the greater part of the effect involves tooth structure, presumably depending on solubility or morphological effects. The fluoride taken up by early caries and the fluoride entering plaque presumably account for the smaller post-eruptive effects although some fluoride is incorporated into enamel after eruption. It seems likely that the surprisingly high level of fluoride in plaque in areas without fluoride in the water and the far from negligible concentration also in all teeth probably exert some restraining effects on caries. In other words, the total value of fluoride cannot be fully measured by comparing the caries incidence in towns with and without fluoride in the water.

The small quantities in saliva, food and drink which provide the fluoride in teeth from areas without fluoride in the water probably have an important protective role but this cannot, of course, be measured as there is no means of knowing how much caries there would be in the complete absence of fluoride.

BIBLIOGRAPHY

For a full list of references to this subject see JENKINS, G. N.: The mechanism of action of fluoride in reducing caries incidence. Int. dent. J. *17*, 552 (1967).
The more importance references are as follows:

1 BRUDEVOLD, F., MCCANN, H. G., and GRØN, P.: Caries resistance as related to the chemistry of the enamel. In "Caries-Resistant Teeth–A Ciba Foundation Symposium, Churchill, London (1965), p. 121.
2 COOPER, V. K., and LUDWIG, T. G.: Effect of fluoride and of soil trace elements on the morphology of the permanent molars in man. N.Z. dent. J. *61*, 33 (1965).
3 DAWES, C., JENKINS, G. N., HARDWICK, J. L., and LEACH, S. A.: The relation between the fluoride concentrations in the dental plaque and in drinking water. Brit. dent. J. *119*, 164 (1965).
4 DOWSE, C. M., and JENKINS, G. N.: Fluoride uptake in vivo in enamel defects and its significance. J. dent. Res. *36*, 816, Abstract 30 (1957).
5 FRAZIER, P. D., LITTLE, M. F., and CASCIANA, F. S.: X-ray diffraction analysis of human enamel containing different amounts of fluoride. Archs oral Biol. *12*, 35 (1967).
6 GRAY, J. A., FRANCIS, M. D., and GRIEBSTEIN, W. J.: Chemistry of enamel dissolution. In "Chemistry and Prevention of Dental Caries", Thomas, Illinois (1962), p. 164.
7 GRØN, P., MCCANN, H. G., and BRUDEVOLD, F.: The direct determination of fluoride in human saliva by a fluoride electrode. Archs oral Biol. *13*, 203 (1968).
8 ISAAC, S., BRUDEVOLD, F., SMITH, F. A., and GARDNER, D. E.: Solubility and natural fluoride content of surface and subsurface enamel. J. dent. Res. *37*, 254 (1958).
9 JENKINS, G. N., EDGAR, W. M., and FERGUSON, D. B.: The distribution and metabolic effects of human plaque fluorine. Archs oral Biol. *14*, 105 (1969).
10 KRUGER, B. J.: Trace elements and dental morphology. J. dent. Res. *41*, 215 (1962).
11 PAYNTER, K. J., and GRAINGER, R. M.: The relation of nutrition to the morphology and size of rat molar teeth. J. Canad. dent. Assoc. *22*, 519 (1956).
12 POSNER, A. S., EANES, E. D., HARPER, R. A., and ZIPKIN, I.: X-ray diffraction analysis of the effect of fluoride on human bone apatite. Archs oral Biol. *8*, 549 (1963).
13 SANDHAM, H. J., and KLEINBERG, I.: The effect of fluoride on the interrelation between glucose utilization, pH and carbohydrate storage in a salivary sediment system. Archs oral Biol. *14*, in press.
14 WALLENIUS, B.: Die Zahnbreite in Relation zum Fluorgehalt im Trinkwasser. Proc. 4th O.R.C.A. Congress, Odontologisk Revy (1957), p. 275.
15 WEISS, S., KING, W. J., KESTENBAUM, R. C., and DONOHUE, J. J.: Influence of various factors on polysaccharide synthesis in Strep. mitis. N.Y. Acad. Sci. *131*, 839 (1965).

Industrial fluorosis

T. L. Vischer, C. Bernheim, C. Guerdjikoff, P. Wettstein and R. Lagier

Fluorides are being investigated for treatment of various bone diseases; they already have their place in prophylaxis of dental caries and may turn out to be an aid in prevention and treatment of osteoporosis.

On the other hand, if intake of fluorides in adults is high and extended over a long period of time, disturbances of bone metabolism follow and characteristic radiological signs appear. High fluoride intake might be due to high fluoride content of water (7), wine (8) or other foodstuffs, or to exposure to fluoride compounds in certain industries (3).

The following study deals with industrial fluorosis: the consequence of long lasting exposure to cryolite and other fluorine compounds in aluminum pot rooms. Simple criteria for radiological diagnosis will be proposed and the implications on the health of the workers discussed.

In the pot rooms of aluminum factories, aluminum oxide (Al_2O_3) is submitted to electrolysis in a bath of cryolite. Before heating, the bath has to be filled with cryolite and aluminum oxide. During electrolysis, cryolite must be added, and also occasionally AlF_3 and NaF. On the surface of the electrolytes a crust is formed which is broken off from time to time. All this was done manually; during the manipulations, dust develops which might be inhaled and swallowed.

Our experience in industrial fluorosis is based on 17 male patients with fluorosis who worked in the pot rooms of aluminum factories over periods of 11 to 46 years. The diagnosis of fluorosis was made through the typical radiological findings, a history of exposure to fluorides over a sufficiently prolonged period of time, elevated fluor values in bone combined with a compatible bone biopsy. All patients were evaluated on the basis of personal history, physical examination and routine laboratory tests. Special care was taken to find other diseases, particularly of the bones and joints. Serum alkaline phosphatase, calcium and phosphate were normal in all patients.

Table 1 gives the age, length of exposure and the fluoride content in bone biopsies of the 17 patients with fluorosis. Fluoride was determined in samples of the iliac crest obtained by open biopsy or later by needle biopsy. Fluor

Table 1. Aluminum pot-room workers with fluorosis (17)

	Mean	*Range*	*Median*
Age (years)	61	52–66	61
Length of exposure (years)	29	11–46	29
Fluoride content of bone (iliac crest; dried and defatted), *gm F'/100 gm bone*	*0 332*	*0 135–0 472*	*0.325*

determinations were done on defatted and dried samples*. Another piece was submitted for histological examination. As the exposure of these workers goes back as much as 46 years, we have no quantitative estimates either about the amount of fluoride they were exposed to, nor about the amounts they absorbed.

RADIOLOGICAL FINDINGS

Increased density of the bone is a generally recognized feature of fluorosis (6) The bone not only has a milky appearance, but its normal trabecular structure is coarsened and later disappears (Fig. 1). This change of structure can be a more reliable sign than "increased bone density", which is often difficult to differentiate from bad X-ray technique. Only 9 of the 17 patients had clear-cut increase in density or change of structure of the bone on X-ray films of the pelvis and lumbar spine

Alterations of the spine were found in all of our cases (Figs. 2, 3, 4), with ossification of ligaments and outgrowth of bony spurs which often form bridges from one vertebra to another. These lesions might have clinical implications, but are not pathognomic, as similar alterations can be found in other patients They occur with increased frequency and thus belong to the well recognized signs of fluorosis (6, 9).

The frequent lack of increased density or derangement of trabecular structure of bone in our cases and the nonspecificity of the alterations of the spine make both of these changes bad criteria for the diagnosis of fluorosis.

The more peripheral findings of exostosis, apposition of new bone, ossification of ligaments and tendon insertions and metastatic, aberrant growth of new bone seem much more specific and constant.

Exostosis can be quite bizarre in shape (Fig. 5) and are frequently found. Apposition of new bone, especially on the long bones, is frequent and occurs

* We wish to thank Prof. D MONNIER, Laboratoire de chimie analytique, University of Geneva, for the fluoride determinations.

Fig. 1. Pelvis with increased density of bone and disappearance of trabecular structure.

2 3

Figs. 2, 3. Lumbar and dorsal spine: ossification of anterior longitudinal ligament and extensive spur-formation, leading to bony bridges between vertebrae.

Fig 4. Cervical spine: gross spur formation.

Fig 5. Exostosis of the left femur.

Fig 6. Forearm apposition of new bone, ossification of interosseous membrane, ossification of tendon insertions

5

6

Fig 9. Free osseous bodies at elbow joint, ossification of tendon insertions

often together with ossification of the interosseous membranes (Fig 6). New formation of aberrant bone, without visible bridge to the other bones, appears to be a constant feature (Fig. 7) Perhaps this develops after breaking off of small exostosis. In one case (Fig. 8) an aberrant piece of bone developed after surgical bone biopsy On a film taken early after biopsy, there was no trace of bone in the tissue. Small independent bone particules were the most frequently present near or in joints, such as elbows, knees and ankles (Fig 9) Care must be taken here not to confuse them with normal accessory bones.

Table 2 shows these radiological findings more systematically. The most constant lesions were found around the elbows. In the knees and ankles, the consequence of trauma as well as normal, accessory bones are more often seen in a general population. Therefore, it seems that X-ray films of forearms and elbows would be the best way to screen a large population for fluorotic bone changes.

Table 2. Radiological findings in industrial fluorosis*

1. Increased density and/or altered structure (pelvis, lumbar spine)	9 / 17
2. Bony bridges between vertebrae	
Cervical spine	1 / 16
Dorsal spine	10 / 17
Lumbar spine	5 / 17
3. Ossifications of interosseous membranes, bony appositions	
Forearms	12 / 14
Lower legs	10 / 16
4. Free osseous bodies, ossification of tendon insertions	
Elbows	11 / 12
Knees	8 / 14
Ankles	9 / 13
5. Alterations of both elbows and forearms	10 / 12
Alterations of both ankles and lower legs	6 / 12

* Number of positives from patients with adequate x-rays.

HISTOLOGICAL FINDINGS

Histological examination revealed bone remodelling with increased density and modification of mineralisation: osteoid seams are present and some cement lines are thickened and appear basophilic, as do certain bone areas, especially those around osteocytes. This remodelling does not seem very active; it expresses itself by the presence of endosteal osteoblasts and areas of fibrous osteoclasia. This picture which is seen in classical cases of fluorosis was observed in 4 of 11 biopsies thus examined; the other biopsies did not show bone remodelling but only disturbances of mineralisation. In one case the bone biopsy appeared practically normal.

CLINICAL FINDINGS

Most authors agree that chronic fluorosis can cause musculoskeletal discomfort and pain (4, 5), despite the fact that well documented cases of fluorosis in patients without any clinical symptoms have been published (9). Most of the patients with chronic industrial fluorosis are elderly persons who have been engaged in manual labour. Little information is available about the natural incidence of musculoskeletal symptoms and degenerative changes in such populations. In addition, our patients consist of a selected group, chosen for suspicion of fluorosis and for symptoms. Only a proper epidemiological study could elucidate the problem of wether persons of this

age and occupational background with chronic fluorosis have significantly more clinical symptoms in contrast to a comparable group. In industrial fluorosis, there exists only one epidemiological survey done in Forth Williams, an aluminium factory in Scotland (1). No increased incidence of symptoms was found in the workers with the highest exposure to fluoride as compared with the other workers of the factory. Only one case in this study is comparable in severity with our cases, judging by the X-ray reports given in the paper.

All but one of the 17 patients complained of vague pains and stiffness in the lower and upper extremities, shoulders, neck and lower back. In none of the cases could another disease of the bone or of the joints be found, except arthrotic lesions. The relation of fluorosis to arthrosis is not clear. Some years ago, the hypothesis was suggested that degenerative changes in cartilage might be the consequence of alterations in the subchondral bone (2). This seems to be a good possibility in arthrosis found after aseptic necrosis or in PAGET's disease. ZIPKIN et al. (10) examined this problem in mice given fluoride over a period of 15 months No increase in arthrotic changes was found; however, mice do not have the same strain on weight bearing joints and might not be as susceptible to fluoride as man. We found a rather high incidence of arthrotic changes in our patients and hope to evaluate this problem in an epidemiological study.

There is no doubt that the alterations of the spine can lead to symptoms. Radicular syndromes have been well documented in India. Three of our patients had clinical evidence of it. Decreased motility of the spine was found in all patients Back pain in general is due in a large part to bad statics and posture, patients with severe alterations of the spine can experience little or even no pain if they can avoid straining their discs and ligaments, watching their posture and doing exercises. Of course a healthy back supports much more strain than a back with alterations of the spine and the ligaments. If fluorotic lesions are present, especially on the cervical and lumbar spine, they probably can cause symptoms.

The high incidence of fluorotic changes on x-rays such as free osseous bodies and ossification of tendon insertions in and near joints raises the question of wether there are more clinical symptoms in these joints. Symptoms are defined here as pain and stiffness in or near the joint with or without limitation of motion. Evaluation of such symptoms is difficult, for pain and joint stiffness are often poorly described by the patient. Pain in the knee may be due to a lesion in the hip, or pain in an elbow due to that in the shoulder. Only patients with adequate X-ray films of the joint in question are included. this might bias the results Also, several patients had accidents involving

Table 3. Correlation of pain and limited range of motion with fluorotic x-ray changes*

	Number	*X-ray changes*		
		present	*absent*	
Knees	9	6	3	with symptoms*
	5	2	3	without symptoms*
	14	8	6	total
Ankles	7	6	1	with symptoms
	6	2	4	without symptoms
	13	8	5	total
Elbows	6	5	1	with symptoms
	6	6	0	without symptoms
	12	11	1	total

* As defined in the text.

these joints in the past. Table 3 is therefore only an attempt to correlate x-ray findings with clinical symptoms. The data suggest that the correlation is best on weight bearing joints. The remark made before about checking with an epidemiological study also applies here; correlation does not necessarily indicate a causal relationship.

We might add here that 6 out of the 17 patients have full working capacity and in those who have some degree of invalidity, other diseases and causes contribute to it.

SUMMARY

Chronic fluorosis alters bone structure, leads to odd exostosis, to osseous appositions, to ossification of ligaments and tendons and their insertions. This can cause pain and discomfort. Radiological examination usually leads to the correct diagnosis; the most constant changes were found in films of elbows and forearms. Such films may be useful in the diagnosis of industrial fluorosis and perhaps for detecting undesirable effects of prolonged fluoride therapy. It seems clear that manifestations of fluorosis in adults appear only after prolonged exposure at high dosage. If signs of fluorosis are present, they may lead to symptoms of the osteoarticular system. Therapy with high dosage fluoride should be monitored carefully.

LITERATURE

1 Agate, J. N , ed.: Industrial fluorosis. Medical research council memorandum No. 22, London, His Majesty's Stationery Office (1949)

2 Johnson, L. C.: Histogenesis and mechanisms in the development of osteofluorosis. In: Hodge, H. C., and Smith, F. A.: Fluorine Chemistry, vol. IV (ed. by J. H. Simons) Academic Press, New York and London (1965).

3 Largent, E J.: Fluorosis. Ohio State University Press, Columbus, Ohio (1961).

4 Peperaorn, Köhling: Arbeitsschutz *3*, 64 (1944), quoted after: Bredemann, G : Biochemie und Physiologie des Fluors, Akademie-Verlag, Berlin (1956).

5 Roche, L , Ravault, P. P , Vignon, G., Lejeune, E , Maîtrepierre, J., and Lambert, R.: Deux nouvelles observations d'ostéopetrose fluorée Arch. Mal. Prof. *21*, 356 (1960)

6 Roholm, K.· Fluorine intoxication. H. K. Lewis, London (1937)

7 Sing, A., Jolly, S S , and Bansal, B C : Skeletal fluorosis and its neurological complications. Lancet *I*, 197 (1961)

8 Soriano, M , and Manchon, F · Radiological aspects of a new type of bone fluorosis, periostitis deformans. Radiology *87*, 1089 (1966).

9 Stevenson, C. A , and Watson, A R.: Fluoride osteosklerosis. Amer. J. Roentg *78*, 13 (1957)

10 Zipkin, I , Sokoloff, L., and Frazier, P. D · A study of the effect of fluoride on bone and osteoarthritis in mice. Isr J. Med Sc. *3*, 719 (1967)

Hydric fluorosis in Punjab (India) *

S. S. JOLLY

INTRODUCTION

The chronic toxic effects of fluoride on the skeletal system have been described from certain geographical regions of the world where drinking water contains excessive quantities of natural fluoride. This form of chronic intoxication was first described in India from the State of Madras as early as 1937 and is called hydric fluorosis (1). Subsequently, cases of hydric fluorosis have been sporadically reported from many other parts of the world, particularly from China (2), Japan (3), South Africa (4), North Africa (5), Argentina (6), Persian Gulf (7), Saudi Arabia (8), United States (9, 10), Canada (11) and Europe (12, 13).

The Department of Medicine, Medical College, Patiala, has been actively engaged in the epidemiological, clinical and biochemical studies of endemic fluorosis for the last nine years. These studies have been carried out in Punjab which is one of the most highly endemic areas in the world. Extensive data on dental, skeletal and neurological aspects of fluorosis have been collected and have been fully reported in our earlier studies (14–20). The object of the present communication is to summarize the results of our studies so far.

MATERIAL AND METHODS

The data to be presented in this paper are based on extensive epidemiological surveys carried out in some parts of the Punjab over the last few years. Punjab is one of the northern states of India and the effected areas lie in its southern and south-western parts. The soil is very sandy in these areas and it has a hot and dry climate with the temperature exceeding 110°F in summer.

* This work was supported with a grant from the Indian Council of Medical Research.

Particular attention was paid to the condition of the teeth and skeletal system of the residents of the affected areas. Dental surveys have been carried out in 358 villages of Punjab and children between age group 5 to 17 years were examined for dental mottling and characteristic dental pigmentation.

Besides the dental surveys, ten villages from endemic fluorotic areas of Punjab with different fluoride concentrations in the drinking water were selected to assess the effect of various factors on fluoride intoxication Children and adults of these villages were subjected to a thorough clinical and radiological examination. Interosseous membrane calcification was taken as a definite radiological index of skeletal fluorosis.

In addition to the cases of dental and skeletal fluorosis studied at the time of epidemiological surveys, about 100 cases of crippling fluorosis were hospitalized. This included a majority of those cases which had the neurological complications of fluorosis. The biochemical determinations in the hospitalized group included urea, sugar, proteins, calcium, inorganic phosphorus, alkaline phosphatase and a study of cerebrospinal fluid, wherever it could be obtained.

done by the Scott-Sanchi method (21) and in the other biological fluids by the methods laid out by the Indian Council of Medical Research (22). Calcium balance studies and estimation of serum enzymes were also included in the later part of this study.

OBSERVATIONS

The toxic effects of the fluoride ion may produce widespread physiological and pathological alterations, although it predominently involves the teeth and the skeletal system. It is proposed to discuss the toxic manifestations as follows

I. Dental manifestations.
II Skeletal changes
III Neurological complications.
IV Crippling fluorosis.

DENTAL FLUOROSIS

Mottled enamel or dental fluorosis is a well recognized entity (23, 24) and is one of the first and earliest visible signs of an excessive intake of fluoride

during the period of dental eruption. The dental changes are divisible into three grades (25):

Grade I White opacities or patches on the enamel and very faint yellow lines across the enamel.

Grade II A distinct brown stain.

Grade III Considerable pitting all over the enamel.

The most striking change in our experience is the typical golden to brownish discoloration of the central and lateral incisors. However, the gross alteration of dentition, discolouring, pitting and chipping is very striking and can be diagnosed very easily.

About forty six thousand children were examined in 358 villages of Punjab. The incidence of dental involvement as correlated to fluoride concentration is tabulated in Table 1.

Table 1

No. of villages	*Maximum concentration of water fluoride (ppm)*	*Incidence of dental mottling (percent)*
210	1.4	0–10
96	2.3	10–30
52	above 2.3	above 30

The incidence of dental fluorosis was found to rise with increasing fluoride concentration but there was no rigid linear relationship between fluoride concentration and the incidence of dental involvement. In the nine villages selected for special study, the incidence of dental mottling in children and adults is given in Table 2.

Table 2. Incidence of mottling in the villages (Figure in brackets indicate total cases examined)

Name of the village	*Fluoride concentration (mean) (ppm)*	*Incidence of dental mottling in children (%)*	*Incidence of dental mottling in adults (%)*
Gharachaon	1.4	22.6 (124)	13.8 (87)
Laduwala	2.4	30.6 (49)	60.2 (74)
Dharpai	3.0	24.5 (57)	47.6 (107)
Bhudipura	3.0	55.9 (34)	31.2 (64)
Rajthal	3.3	47.0 (133)	10.0 (160)
Sanghera	3.6	27.4 (317)	49.4 (154)
Ramjana	5.0	52.7 (93)	56.6 (90)
Gangiculabsingh	8.5	81.4 (43)	55.6 (58)
Khara	9.7	66.0 (50)	70.7 (232)

The incidence of dental fluorosis in a locality with mean fluoride concentration of 1.4 ppm was found to be about 22.6% and to rise with increasing fluoride concentration.

SKELETAL FLUOROSIS

During the intensive epidemiological survey of the villages in Punjab, we had the opportunity of examining 1065 cases of skeletal fluorosis, the largest series reported so far in the literature. A detailed examination was also done on cases which were admitted to the hospital from time to time for the last ten years. Table 3 gives the details of the cases of skeletal fluorosis.

Table 3

	Total	*Percentage*	*Males*	*Females*
No. of cases of skeletal fluorosis (detected on radiological examination)	1065			
a) latent or subclinical cases with no symptoms	210	19.7%		
b) symptomatic	855	80.3%		
without crippling	624	58.6%	70%	30%
with crippling deformities	142	13.3%	92%	8%
with neurological complications	89	8.4%	94%	6%

Whereas dental fluorosis is easily recognized, the early skeletal involvement is not clinically obvious until the advanced stage of crippling fluorosis. However, radiological changes are discernible in the skeleton at a much earlier stage and provide the only means of diagnosing the early and relatively asymptomatic stage of fluorosis. These cases are usually young adults whose only complaints are vague pains noted most frequently in the small joints of the hands and feet, the knee joints, the legs and spine. Such cases are frequent in endemic areas and are misdiagnosed as rheumatoid [illegible] or [illegible]

[illegible] development of kyphosis. There is difficulty in walking, partly due to stiffness and limitation of movements of various joints, and partly due to a neurological deficit in advanced cases.

The various skeletal changes in the endemic fluorosis are best described under following headings:

1. Gross changes in the skeleton.
2. Radiological changes.
3. Histopathology.
4. [illegible]
5. [illegible]

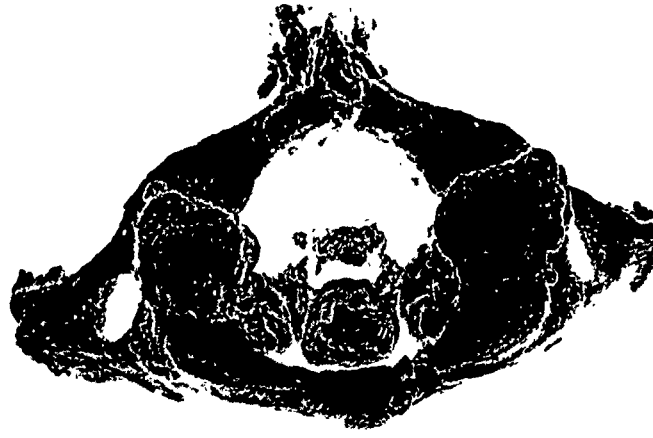


Fig. 1. Third cervical vertebra showing a huge exostosis projecting into the spinal canal.

1. Gross changes in the skeleton

The gross changes in the skeleton in cases of endemic fluorosis are quite distinctive and characteristic. The excessive quantities of fluoride which are ingested are deposited in the skeleton over the years. We had a unique opportunity to study the completely macerated skeleton of an individual who was living in an endemic area with a fluoride content of 9.5 ppm (16). All the bones were observed to be heavy and irregular and had a dull colour due to irregular deposition of fluoride. The sites of muscular and tendinous insertions were rendered abnormally prominent by excessive periosteal reaction with development of multiple exostoses. Irregular bone was laid down along the attachment of muscles and tendons in the extremities as well as in joint capsules and interosseous membranes. The latter is particularly helpful as a diagnostic feature in doubtful and borderline cases where the density of the bones is not markedly increased.

Changes are detected in the spine with calcification of various ligaments, particularly the ligamenta flava, intertransverse and interspinous ligaments, resulting in marked osteophytes. The vertebral bodies are larger than normal and show marked lipping. The vertebrae show altered proportions and measurements in all the planes, but the striking abnormity is the gross reduction of anteroposterior diameter of the spinal canal (Fig. 1). In one of our cases it was reduce to 2 mm at the level of the third and fourth cervical vertebrae. Since the average anteroposterior diameter of the spinal cord in the cervical enlargement is 8 mm and the bulge of ligamentum flavum has

Fig. 2. Narrowing and irregularity of the intervertebral foramina.

also to be accounted for, it is evident that the compression of the cord is almost inevitable. The vertebrae are fused at many places which explains the marked limitation of movements and the resemblance of the disease to spondylitis ankylopoietica. The intervertebral foramina are narrowed and rendered irregular which explains the presence of radicular manifestations

foramen magnum are also rendered irregular and narrow due to the projection of osteophytes The other smaller foraminae in the skull are usually not altered, thus explaining the absence of cranial nerve involvement in advanced cases of endemic fluorosis.

The ribs are large, with rough surfaces and osteophytes projecting along the attachments of muscles, membranes and ligaments.

The other bones, including those of the limbs, the sternum and the mandible, have many prominent osteophytes at the attachments of ligaments, membranes, tendons and muscular insertions, thus making the various markings and ridges thick and prominent. The interosseous membranes between tibia and fibula and between radius and ulna are ossified to a

variable degree in most of the cases. There is thickening and calcification in most of the ligaments and many of the capsular attachments, such as the sacroiliac and sacro tuberous ligaments. The thyroid cartilage is also calcified in most of the cases.

2. Radiological changes

The radiological changes of skeletal fluorosis are diagnostic (26, 27). The most pronounced changes are seen in the vertebral column particularly in the cervical and lumbar region. Osteosclerosis and irregular osteophyte formation is noted in the vertebral body, the transverse and spinous processes, the pedicles and laminae. Beak-like lipping and the chalky white ground glass appearance of the entire vertebral column are the characteristic radiological features. There is calcification of the intervertebral ligaments. As a result of irregular exostoses, there is encroachment on the intervertebral foramina and the spinal canal. Next to the spine, the osteosclerosis is most evident in the pelvis along with calcification of sacrotuberous and sacrospinous ligaments. Irregular periosteal bone formation is observed along tendons, fascial, and muscular attachments including the interosseous membranes of the forearm and legs, linea aspera, the deltoid tuberosity, the lower margin of the ribs, the attachment of the achilles tendon, the tibial tubercle and the greater trochanter of femur. Skiagrams of the chest reveal a peculiar contrast of the marble white bony cage with radiolucent lungs. The changes in the skull are not very striking although there is thickening of the vault with sclerosis near the suture lines. The sella turcica and the nasal sinuses are normal and there is no significant narrowing of the basal foramina.

istopathology

Although there are many histopathological reports on experimental fluorosis (28) the data in human intoxication is scanty. Our observations are based on bone biopsies obtained either from the tibia or iliac crest or from the spine at the time of laminectomy. In general, the compact bone shows disordered lamellae and an enlarged, poorly formed haversian system. In the spongy bone, areas of osteoid tissue are found among well formed trabeculae. Some of the irregular deposits of osteoid tissue extend into the attached muscle. The bone trabeculae are very dense in places and contain a considerable amount of calcium. The areas around the vascular spaces stain deeply with eosin. In some cases the muscular attachments to the bones may show areas of irregular calcification.

The most obvious effect of persistent high fluoride ingestion is stimulation of osteoblastic activity resulting in the production of exostoses and the

calcification of tendons, ligaments and occasionally muscles. Some bones are more prone to exostoses formation than others; the vertebrae, ribs and pelvis for example are more susceptible than the long bones. In an advanced case, however, the entire skeleton is involved.

Skeletal fluorosis has been likened to a number of bone diseases The dense radiographic picture of the skeleton has resulted in comparison with osteosclorosis; the presence of broad osteoid seams has suggested osteomalacia; the way in which bone formation may proceed side by side with bone destruction is reminiscent of PAGET's disease and the often extensive resorption points to osteoporosis. Certainly, fluorotic bones can exhibit signs common to each of these conditions but a unique distinction is the presence of high levels of fluoride in the bone.

4. Chemical composition

There are very few studies of the chemical composition of bones in human cases of chronic fluorine intoxication (29, 30) although considerable literature

nisms In the first, fluoride exchanges with hydroxyl on the surface of existing crystals. The second mechanism is that of new bone formation by osteoblastic and osteoclastic activity. However, the precise mode by which the fluoride exerts its deleterious effects is not known. It is probable that there are initial changes in the chemical composition and deposition of bone salts in the organic matrix, possibly mediated by altered enzyme reactions. ROHOLM believed that the fluoride was probably deposited in the form of calcium fluoride along with calcium phosphate of the bone but the work of WEIDMANN, WEATHERELL and WHITEHEAD (31) demonstrated a decrease of

studied fluoride deposition in human bones after prolonged ingestion of fluoride in drinking water and came to the conclusion that the mean concentration of fluoride in the various bones was correlated with the fluoride level of drinking water up to 4 ppm and that there is no indication in their data that these human calcified tissues approach their theoretical capacity of about 3 5%, fluoride. A concentration of fluoride as high as 0.548% in the dry, fat free bone and 1 080% in the bone ash may be present without

700 mg/

100 Gm dry weight against a normal of 20 to 30 mg in a person from a non fluorotic area. It has been pointed out that in human beings, osteosclerosis would be evident in a small proportion of individuals with skeletal concentrations of fluoride of the order of 6000 ppm (32).

5. Deformities and crippling fluorosis

This advanced stage of fluoride intoxication results from the continuous exposure of an individual to 20–80 mg of fluoride ion daily over a period of 10–20 years. Such heavy exposure is associated with a level of at least 10 ppm in the drinking water supply. In the areas surveyed by us this level was not only common but was often exceeded. Moreover, besides the fluoride ingested in the water there were additional sources of ingestion such as in vegetables grown in the fluorotic soil and the processing and cooking of food in the water contaminated with fluoride ion. Therefore, it was not surprising that cases of crippling fluorosis are seen in such numbers in endemic areas of Punjab.

The crippling deformities are due partly to mechanical factors and partly to the immobilization necessitated by pain and paraplegia. The commonest deformities are kyphosis, flexion deformity of the hips, flexion deformity of the knees and fixation of the chest in the position of inspiration due to calcification of the cartilage. The advanced picture of crippling fluorosis is strikingly uniform. The quadriplegic patient bent with kyphosis and with markedly restricted movements of his spine with contractures of hips and knees provides a grim picture of the result of excessive fluoride intake. Due to the extreme fixation of the spine, the body moves as a single unit with each attempt to straighten any portion of it.

NEUROLOGICAL MANIFESTATIONS OF FLUOROSIS

It had been reported in our earlier studies that some cases of skeletal fluorosis develop the neurological complications of radiculomyelopathy due to compression of the spinal cord and roots as a result of irregular bone deposition in and around the spinal canal. This has been convincingly demonstrated in a macerated skeleton by us (16). So far we have studied 62 proved cases of skeletal fluorosis with neurological manifestations. This is the largest series in the world. The details of neurological manifestations are shown in Table 4. It is worth emphasizing that only 5 cases of neurological fluorosis were seen in women against 57 observed in men.

Table 4 A summary of 62 cases with neurological manifestations

Type of neurological lesion	*Males*	*Females*
Cervical radiculomyelopathy	31	–
Cervical radiculomyelopathy with deafness	1	–
Cervical radiculomyelopathy with cerebellar involvement	1	–
Purely cervical myelopathy	4	1
Cervico-dorsal myelopathy	2	1
Dorsal myelopathy	12	3
Cervical radiculopathy	1	–
Peripheral neuritic type	3	–
Fluorosis associated with cerebrovascular accidental*	2	–
Total	57	5

* These cases are probably due to compression of the vertebral artery as it is coursing through the cervical spine

It will be noticed that most of the cases had a pattern of radiculomyelopathy although a few cases had the interesting features of peripheral neuropathy The involvement of the vertebral artery as it courses through the cervical spine explains the presence of cerebellar symptomatology.

The evidence so far points to the fact that the neurological manifestations are entirely due to compression and are not due to the toxic effect of fluoride on the nervous system.

BIOCHEMICAL DATA

A. Fluoride in water sample

About 2500 samples of water were examined from different regions of the State including the endemic and non-endemic areas The fluoride content varied considerably from less than 1 to 16 ppm. It was therefore not precisely possible to define the safe lower limit.

Table 5. Incidence of skeletal fluorosis

Name of village	*Concentration of fluoride in water (ppm)*	*Incidence of skeletal fluorosis*	*Crippling fluorosis*
Gharachon	1.4 (0.9–2.5)	2.4 (82)	— ve
Laduwala	2.4 (1.0–5.5)	23.0 (74)	— ve
Dhapai	3.0 (1.1–5.5)	19.6 (107)	— ve
Bhodipura	3.0 (1.3–5.2)	42.2 (64)	+ ve
Rajthal	3.3 (0.5–6.5)	10.0 (160)	— ve
Bhikhi	3.3 (1.0–5.9)	45.6 (160)	+ ve
Sanghera	3.6 (1.1–5.8)	33.1 (154)	+ ve
Ramuana	5.0 (1.5–11.5)	60.0 (90)	+ ve
Ganjigulab Singh	8.5 (3.7–14.0)	58.9 (56)	+ ve
Khara	9.7 (6.0–16.2)	80.7 (232)	+ ve

A study of Table 5 shows that the incidence of skeletal fluorosis is practically nil at a mean concentration of 1.4 ppm but the incidence rises with increase of fluoride concentration in water. Similar to dental fluorosis, the concentration of fluoride alone is not responsible for the incidence of skeletal fluorosis.

In our study, we came across two interesting observations as will be evident from a study of Table 5. We found cases of crippling fluorosis in endemic areas having a mean water fluoride content as low as 3 ppm. Secondly, whereas in some villages with a mean fluoride concentration of 3 and 3.3 ppm there were cases of crippling fluorosis, in two other villages the same concentration did not result in crippling fluorosis. This is possibly related to the protective role of other factors such as the calcium and magnesium content of the water in these villages (33, 36).

B. Fluoride estimations in body fluids and tissue

In addition to simple biochemical estimation of water fluoride, other estimations of urinary, blood and bone fluorides were done in the hospitalized group of patients and their results are illustrated in Table 6.

Table 6. Fluoride content of blood, urine and bones

	Total number of estimations	*Range*	*Mean*	*Normal*
Blood (mg%)	42	0.05–0.8	0.28	traces only
Urine (mg%)	26	0.10–1.80	0.429	less than 0.185
Bone (mg/100 Gm boneash)	20	70–700	318.7	110 ± 20

C Miscellaneous biochemical data

In the hospitalized cases of fluorosis, a number of other biochemical investigations were done to look for any other toxic effects. The levels of

The parathyroid function tests, the thyroid function tests and the adrenocortical assessment were within normal limits.

FACTORS INFLUENCING TOXICITY

1. Fluoride content of drinking water. It is universally agreed that fluoride ingestion produces toxic effects, but the concentration which may produce deleterious effects is the subject of controversy. The minimal threshold has not yet been established definitely. In some studies from India, a lower concentration of fluoride has been shown to be associated with well marked fluorosis The intensive survey of the villages done by us shows that concentrations of fluoride ranging from 0.9 ppm to 2.5 ppm are associated with an incidence of only 2 4% skeletal fluorosis, but that crippling fluorosis was seen in some of the villages with water fluoride range of 1 3 to 5.2 ppm. The belief that the incidence of skeletal fluorosis is dependent only on the fluoride concentration is falsified when two villages, Rajthal and *Bhikhi*, are compared (Table 7) Both these villages have practically the same fluoride concentration, but they show a marked difference in the incidence of skeletal fluorosis In the village of Bhikhi, a large number of crippling fluorosis cases were observed but not a single similar case was detected from the village Rajthal, which suggests the existence of some other factors beside water fluoride concentration alone.

2 Duration of fluoride exposure The duration of exposure to fluoride intoxication has a definite influence on the development of endemic fluorosis in that with a similar fluoride concentration the incidence is found to increase with age In villages having a lower fluoride concentration, skeletal fluorosis is detected in a higher age group as compared to those ingesting high fluoride concentration where it is seen at a comparatively younger age.

3 Sex and occupation also have some influence on the development of endemic fluorosis, particularly in relation to the severe varieties such as neurological and crippling fluorosis because there were only 5 females as compared to 57 males in hospitalized cases of neurological fluorosis. The disease is far more common in labourers and farmers who have to do hard manual work and also carry heavy loads on the head and this perhaps is

one of the factors that accounts for the severer type of disease in men and the high incidence of neurological and crippling fluorosis.

Another contributory factor may be that a woman's stay in an endemic area is divided as she has to migrate to another village after marriage where the fluoride content of the water supply may be different.

4. Chemical composition of the water (other than fluoride). Besides the fluoride content, a number of other constituents in the water are also important, such as the calcium hardness, the magnesium hardness and the alkalinity. We observed that fluoride concentration bears an inverse relationship to total hardness and calcium hardness. In areas having low fluoride content, the water is harder. The incidence of endemic fluorosis depends, to a great extent, on the hardness of water. This point is illustrated by comparing the analysis of water constituents from the villages of Rajthal and Bhikhi as in Table 7.

Table 7. Chemical constituents of water

Village		*Fluoride (ppm)*	*Total hardness*	*Calcium hardness*	*Magnesium hardness*	*Incidence of skeletal fluorosis*
Rajthal	Mean	3.3	601	358	344	
	Range	0.5–6.5	308–1136	120–800	108–506	10.0%
	S.D.	0.5	61	57	40	
Bhikhi	Mean	3.3	136	56	80	
	Range	1.0–5.9	32–312	6–190	26–198	45.6%
	S.D.	0.3	20	98	10	

These two villages have the same fluoride content but the incidence of endemic fluorosis shows a wide variation. Other factors such as nutritional status, climatic effect, duration of fluoride exposure, sex, profession, etc., were identical in the two villages, the only difference being calcium hardness and magnesium hardness. In America, most of the water containing fluoride is hard water and the fluoride/hardness ratio is more than one in 500. In our study the fluoride/hardness ratio was much less.

It is thus clear that both the calcium and magnesium content of water have a protective influence on the absorption of the fluoride and its subsequent deposition in the skeleton.

In India, lower toxic limits of fluoride may be related to less hard water as shown in the analysis of water constituents in some of the endemic areas.

5. Nutritional factors. It has been alleged that the higher incidence of endemic fluorosis in India is partly related to malnutrition because with a

similar fluoride concentration, no case of fluorosis has been detected from other parts of the world such as Texas Our studies have been done in Punjab which is one of the best nourished states in India, and it is in this very state that the incidence of fluorosis is highest. However, this needs further elucida-

observed only from those areas where the soil is sandy and there is a hot, dry type of climate. The average temperature during the summer is above 100°F and the rainfall is rather scanty. Moreover, cases of fluorosis have been observed only from those village where the subsoil water was rather superficial. In deeper wells the fluoride content of water is not as high and

fluoride may be ingested from the food grown in soil rich in fluoride, from tea and from wines (35) Recently, high levels of fluoride have been shown to be present in the cooking salt in Punjab and in turmeric which is used as an adjuvant in cooking all over India. We have still not precisely evaluated these factors in our endemic areas, but it is quite possible that these factors may aggravate fluoride intoxication because the villagers of Punjab consumes a lot of tea and alcoholic drinks

SUMMARY

An epidemiological, clinical and biochemical study of hydric fluorosis is described from Punjab (India) This condition is due to chronic fluoride intoxication resulting from ingestion of large quantities of fluoride in the drinking water spread over a number of years. It produces a well defined disorder affecting the teeth, the skeletal system and secondarily the nervous system

The results of dental surveys in 358 villages are described and an effort has been made to correlate it with water fluoride levels

The details of skeletal changes as observed by gross examination, radiological appearances, histopathological changes and alterations of chemical composition are described This is the largest series of skeletal fluorosis in the literature.

The pattern of neurological deficit conforms to a radiculomyelopathy and is due to compression of spinal cord and nerve roots

Significantly high levels of fluoride are described in the blood, urine and bones of these patients and in the drinking water samples of the affected population, It has not been possible so far to define the safe lower limit of fluoride in the drinking water.

There is no significant alteration in the thyroid, adrenalcortical and parathyroid functions. The serum enzymes are mostly normal except alkaline phosphatase which is raised.

The role of various factors causing fluoride toxicity is discussed, in particular the protective effect of calcium and magnesium ions in hydric fluorosis.

REFERENCES

1 Shortt, H. E., McRobert, G. R., Barnard, T. W., and Nayar, A. S. M.: Endemic fluorosis in the Madras presidency. Ind. J. Med. Res. *25*, 553 (1937).

2 Lyth, O.: Endemic fluorosis. In: Kweichow, China, Lancet *1*, 233 (1946).

3 Takamori, T.: Recent studies on fluorosis. Tokushima J. Exptl. Med. *2*, 25 (1955).

4 Ockerse, T.: Endemic fluorosis in South Africans-Pretoria (Thesis University of Witwatersrand). Abstract in Bull. Hyg. *20*, 20 (1945).

5 Murray, M. M., and Wilson, D. C.: Fluorosis and nutrition in Morocco. Dental studies in relation to environment. Brit. Dent. J. *84*, 97 (1948).

6 Raffaele, J. F.: La fluorosis, EI Atenes, Buenos Aires (1944).

7 Azar, H. A., Nuchco, C. K., Bayyuk, S. I., and Bayyuk, W. B.: Skeleta sclerosis due to chronic fluoride intoxication. Cases from an endemic area of fluorosis in the region of Persian Gulf. Annals of Int. Med. *55*, 193 (1961).

8 EL Tanner, M. D.: Motiling of enamel in Mecca and the Arabian Penisula: a survey and research study carried out in Saudi Arabia. Amer. J. Public Health *49*, 45 (*1959*).

9 Waldbott, G. L.: Physiologic and hygienic aspects of absorption of inorganic fluorides. Arch. Environ Health *2*, 59, 155 (1961).

6 Zipkin, I., McClure, F. J., Leone, N. C., and Lee, W. A.: Fluorine deposition in human bones after prolonged ingestion of fluoride in drinking water. Publ. Health Rep. *73*, 721 (1955).

1 Kilborn, L. G., Outerbridge, T. S., and Lei, H. P.: Fluorosis with report of and advanced case. Canad. M.A.J. *62*, 135 (1950).

2 Demol, E. V., and Held, A. J.: Fluorine and general health. State of health of the native and long term residents of the village of Sembrancher. Schweiz. Med. Wschr. *83*, 362 (1953).

13 Galeazzi, M.: Il fluoro in nature come agents morbigeno. Nota III. Un focolaio di fluorosisumana a Campagnano di Roma. Rendic Ist Sup. Sanita (1947), p. 739.

14 Singh, A., Jolly, S. S., and Bansal, B. C.: Skeletal fluorosis and its neurological complications. Lancet *1*, 197 (1961).

15 Singh, A., and Jolly, S. S.: Endemic fluorosis with particular reference to fluorotic radiculomyelopathy. Quart J. Med. N.S. *30*, 357 (1961).

Significantly high levels of fluoride are described in the blood, urine and bones of these patients and in the drinking water samples of the affected population, It has not been possible so far to define the safe lower limit of fluoride in the drinking water.

There is no significant alteration in the thyroid, adrenalcortical and parathyroid functions. The serum enzymes are mostly normal except alkaline phosphatase which is raised.

The role of various factors causing fluoride toxicity is discussed, in particular the protective effect of calcium and magnesium ions in hydric fluorosis.

REFERENCES

1 Shortt, H. E., McRobert, G. R., Barnard, T. W., and Nayar, A. S. M.: Endemic fluorosis in the Madras presidency. Ind. J. Med. Res. *25*, 553 (1937).

2 Lyth, O.: Endemic fluorosis. In: Kweichow, China, Lancet *1*, 233 (1946).

3 Takamori, T.: Recent studies on fluorosis. Tokushima J. Exptl. Med. *2*, 25 (1955).

4 Ockerse, T.: Endemic fluorosis in South Africans-Pretoria (Thesis University of Witwatersrand). Abstract in Bull. Hyg. *20*, 20 (1945).

5 Murray, M. M., and Wilson, D. C.: Fluorosis and nutrition in Morocco. Dental studies in relation to environment. Brit. Dent. J. *84*, 97 (1948).

6 Raffaele, J. F.: La fluorosis, El Atenes, Buenos Aires (1944).

7 Azar, H. A., Nuchco, C. K., Bayyuk, S. I., and Bayyuk, W. B.: Skeleta sclerosis due to chronic fluoride intoxication. Cases from an endemic area of fluorosis in the region of Persian Gulf. Annals of Int. Med. *55*, 193 (1961).

8 El Tanner, M. D.: Motiling of enamel in Mecca and the Arabian Penisula: a survey and research study carried out in Saudi Arabia. Amer. J. Public Health *49*, 45 (1959).

9 Waldbott, G. L.: Physiologic and hygienic aspects of absorption of inorganic fluorides. Arch. Environ Health *2*, 59, 155 (1961).

10 Zipkin, I., McClure, F. J., Leone, N. C., and Lee, W. A.: Fluorine deposition in human bones after prolonged ingestion of fluoride in drinking water. Publ. Health Rep. *73*, 721 (1955).

11 Kilborn, L. G., Outerbridge, T. S., and Lei, H. P.: Fluorosis with report of and advanced case. Canad. M.A.J. *62*, 135 (1950).

12 Demol, E. V., and Held, A. J.: Fluorine and general health. State of health of the native and long term residents of the village of Sembrancher. Schweiz. Med. Wschr. *83*, 362 (1953).

13 Galeazzi, M.: Il fluoro in nature come agents morbigeno. Nota III. Un focolaio di fluorosisumana a Campagnano di Roma. Rendic Ist Sup. Sanita (1947), p. 739.

14 Singh, A., Jolly, S. S., and Bansal, B. C.: Skeletal fluorosis and its neurological complications. Lancet *1*, 197 (1961).

15 Singh, A., and Jolly, S. S.: Endemic fluorosis with particular reference to fluorotic radiculomyelopathy. Quart J. Med. N.S. *30*, 357 (1961).

Endemic fluorosis in Morocco (darmous): microradiographic study of human bone and teeth lesions

C. A. Baud and A. H. Alami

It has long been known that the teeth of inhabitants and cattle living in the areas of the Moroccan rockphosphate mines characteristically show a mottled enamel; this abnormality was called "darmous". Bony lesions, hyperostosis and osteosclerosis, have been at times revealed by radiography in association with these dental lesions.

It is generally admitted that the darmous is an endemic fluorosis (6). Indeed the natural phosphates of Morocco contain a high percentage of fluorine, which may be a cause of intoxication. However, recently the fluoride origin of the darmous dental lesions has been questioned (15), while certain authors think that the darmous is a specifically dental illness and does not affect the bones (4).

The aim of this research was to determine by means of microradiographic observations if the lesions of the calcified bony and dental tissues are similar to those observed in the endemic fluoroses already known and in the experimental fluorosis.*

MATERIALS AND METHODS

100 human teeth from Khouribga (a phosphate area of Morocco), some of which with adjacent alveolar bone attached, were embedded in methacrylate. Transverse and longitudinal sections were cut, and microradiographs prepared (2).

Fluorine estimations were carried out on dental enamel, rockphosphates, well water from the environment of Khouribga, and tea with fresh mint (Moroccan national drink), by the fluoride specific electrode method (19).

* These studies were supported by grants from the Swiss Academy of Medical Sciences, the Swiss Society of Odontostomatology, the Zyma Company, and the Swiss National Fund for Scientific Research.

Fig. 1. Microradiograph of an enamel ground section. hypomineralized striations. ×175.

RESULTS

In the enamel, hypomineralized striations parallel to the incremental lines are numerous (Fig 1); however, there remains mostly narrow bands of well calcified substance at the amelo-dentinal junction and at the surface.

In the dentine, hypomineralized layers parallel to the contour lines, sometimes extending along the canaliculae (Fig 2), and extensive areas of granular dentine (Fig 3) are seen in some cases; nevertheless, the dentine is apparently normal in many fluorosed teeth which present enamel abnormalities

In the cement, diffuse hypercementosis (Fig. 4) with numerous cementocytes in large lacunae (Fig 5), pronglike excrescences and cementicles (Fig. 6) are often demonstrated..

In the alveolar bone, all the cortical and cancellous tissue has an abnormal "mottled" structure, characterized by an excessive number of osteocytes in large lacunae (Fig 7), and small defects of calcification in the intercellular substance (Fig. 6)

The results of fluoride estimation are shown in Table I, and indicate that rockphosphate is the main fluorine supply, either directly ingested in dust form, or indirectly with drinks, vegetables and other foods

Fig. 2. Microradiograph of a dentin ground section: hypomineralized layers. × 175.

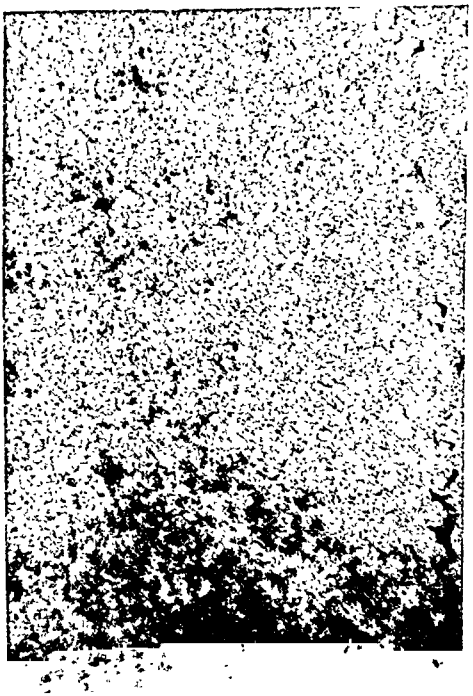


Fig. 3. Microradiograph of a dentin ground section: granular appearance with calcospherites. × 70.

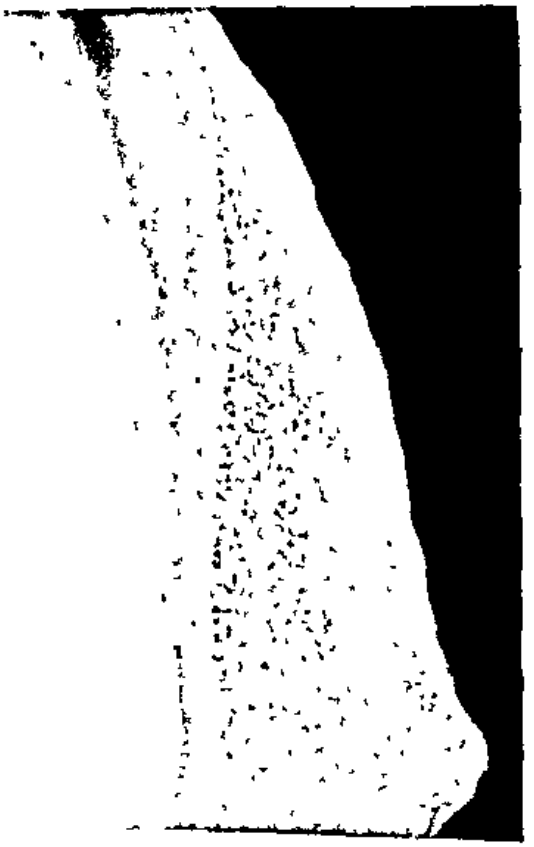


Fig. 4. Microradiograph of a root ground section: diffuse hypercementosis. ×30.

Fig. 5. Mic
ground section numerous large cementocyte lacunae. ×70

Fig. 6. Microradiograph of a ground section through a root and adjacent alveolar bone: pronglike excrescences of the cement (white arrow, at right) and small defects of calcification in the intercellular bone substance (black arrow at left). ×70

Fig. 7. Microradiograph of alveolar bone ground section: numerous large osteocytes lacunae. ×70.

Table 1. Fluoride analyses

Material	*ppm F min*	*ppm F max.*
Surface enamel	1 714	1 960
Rockphosphate from Khouribga	32 727	33 777
Green tea with mint	1.48	1.52
Samples of water drawn up from 9 different wells in the environment of Khouribga	0.32	1.24

DISCUSSION

1. Endemic fluorosis in man

Hypomineralized layers of enamel, parallel to the incremental lines, have been demonstrated by microradiography in human fluorosed teeth (1, 7, 8, 9, 10, 11, 18) In the areas of poorest calcification, the organic matrix is altered or absent, these areas are in fact not only hypomineralized, but also microhypoplastic (8)

the enamel (3) These observations suggest that ameloblasts are more susceptible to fluoride than are odontoblasts, and that the doses of fluoride which produce lesions in human enamel are often too small to damage odontoblasts and dentin.

As for the cement, an extensive hyperplasia of the osteocementum together with calcified bodies in the connective tissue of the periodontal l

of enamel and dentine), [illegible] an excessive number of osteocytes in large lacunae, represents the typical lesion of the fluorosis (16, 21) in the skeleton.

The microradiographic alterations of the darmous are therefore quite similar to that of other human fluoroses

2 Experimental fluorosis

The effects of high fluoride diets on developing enamel and dentin in the incisors of pigs and rats have been extensively described (5, 22, 23) In both tissues, microradiography disclosed abnormalities in the distribution of mineral striations representing alternate layers of relatively normal and deficient mineralization, calcospherites and hypomineralized interglobular

spaces, in the dentin; hypomineralized bands parallel to the incremental lines, in the enamel.

"Slobbers" is a form of chronic fluorosis in laboratory Guinea pigs (12); this disease was caused by the ingestion of pellets of food containing a fine phosphate powder from Christmas Island, of which the fluoride content varied between 1.5 and 3.25%. It is also characterized by hypoplasia and impairment of calcification of enamel, some odontoblast degeneration and impairment of dentine formation, and calcifications in the periodontal connective tissue. Experimental reproduction of this disease was accomplished in exact detail by the extended administration of fluoride (13, 14).

The exact mechanism through which these changes are produced is still uncertain; the electron microscope observations (3, 17) demonstrate that heavy doses of fluoride have a direct effect on the intracellular organelles responsible for the synthesis of proteins and the production of energy, and could well influence not only the quality of the organic matrices, but also the rate of mineralization by impairing active transport of calcium across the cell membrane into the extracellular space.

SUMMARY

The abnormalities in the calcified tissues of the dental organ in human darmous, evidenced by microradiography, consist essentially in:

- hypomineralized layers parallel to the incremental lines in enamel;
- hypomineralized layers parallel to the contour lines, and extensive granular areas, in the dentine;
- diffuse hyperplasia and localized excrescences of the cement;
- "mottled" structure of the alveolar bone.

Such changes are closely related to similar changes demonstrated in the teeth from laboratory animals on high fluoride diets, and in the fluorotic mammalian skeleton. This clarifies the etiology of the abnormalities observed in darmous.

REFERENCES

1 APPLEBAUM, E.: Mottled enamel. Dent. Cosmos *78*, 969 (1936).
2 BAUD, C. A.: Radiographies et microradiographies osseuses quantitatives. Praxis *46*, 329 (1957).
3 BAUD, C. A., and BANG, S.: Fluoride and bone mineral substance. This volume, p. 27.
4 BECMEUR, N., LAMOTTE, L., MASSOTTE, M., et ROUSSON, R.: Darmous et fluoroses. Maroc méd. *30*, 490 (1951).

5 Belanger, L. F., Lotz, W. E., Visek, W. J., and Comar, C. L.: Autoradiographic visualization with Ca^{45} of normal growth of the incisor of pigs and the effect of fluorine feeding. Anat. Rec. *119*, 53 (1954).
6 Drabant, H., Hassar, M., et Klees, L.: Observations cliniques et histologiques sur la fluorose endémique (Darmous) des régions des mines de phosphates du Maroc. Acta stomatol. belg. *60*, 321 (1963).
7 Crabb, H. S. M., and Darling, A. I.: The pattern of progressive mineralisation in human dental enamel. Oxford, Pergamon (1962).
8 Darling, A. I., and Brooks, A. W.: Some observations on the *mottled* enamel of fluorosis. J. dent. Res. *38*, 1226 (1959).
9 Gustafson, A. G.: The histology of fluorosed teeth. *Arch. oral Biol. 4*, 67 (1960).
10 Gustafson, G., and Gustafson, A. G.: Human *dental* enamel in polarized light and contact microradiography. Acta *odontol. scand. 19*, 259 (1961).
11 Gustafson, G., and Gustafson, A. G.: *Imperfections* in enamel structure and their possible relation to caries susceptibility. Proc. 6th ORCA Congr. Pavia 1959, p. 27–33 (1962).
12 Hard, G. C., and Atkinson, F. F. V.: "Slobbers" in laboratory guinea-pigs as a form of chronic fluorosis. J. Path. Bact. *94*, 95 (1967).
13 Hard, G. C., and Atkinson, F. F. V.: The aetiology of "slobbers" (chronic fluorosis) in the guinea-pig. J. Path. Bact. *94*, 103 (1967).
14 Held, A. J., and Grasset, E.: Pulpa- und Dentinschadigungen durch Fluor-Intoxikation bei erwachsenen Meerschweinchen. Dtsch. zahnarztl. Zschr. *7*, 430 (1952).
15 Jeanneret, R.: Über den "darmous", den "gefleckten Schmelz" (*mottling*), die "Dentalfluorose", die "*Schmelzhypoplasien*", die "chronische *Fluor-Toxikose*", die Industrie-Fluorschäden beim Haustier und beim Menschen. Schweiz. Monatsschr. Zahnheilk. *74*, 515 (1964).
16 Johnson, L. C.: Histogenesis and mechanisms in the development of osteofluorosis. In *Fluorine chemistry*, ed. by J. H. Simons, vol. 4, [illegible]
17 [illegible]
18 Newbrun, E., and Brudevold, F.: Studies on the physical properties of fluorosed enamel. J. [illegible]
19 Richar[illegible] fluoride
20 Singh, [illegible], Jhatael, S. S., and Jolly, S. S.: Skeletal changes in endemic fluorosis. J. Bone Jt. Surg. *44B*, 806 (1962).
21 Thiebaud, M., et Baud, C. A.: Traitement des ostéopathies atrophiques par le NaF. Helv. med. Acta *34*, suppl. *48*, 126 (1969).
22 Toda, Y., and Van Huysen, G.: Fluoride and the calcification of the rat incisor. J. dent. Res. *45*, 1567 (1966).
23 Yaeger, J. A.: The effects of high fluoride diets on developing enamel and dentin in the *incisors* of rats. Amer. J. Anat. *118*, 665 (1966).

Fluorosis in cattle

W. LEEMANN

Fluorosis in domestic animals, when considered from an economic point of view, is only important in cattle and sheep, whereas in the other species, it is relatively insignificant. In general, chronic intoxications have been observed more frequently, acute poisonings being rather exceptional. As the symptoms in sheep and cattle are similar, those described here in connection with the latter species can be readily applied to both.

The first reports on diseases in cattle associated with the industrial emission of fluorides appeared during the 19th century (ALTHER). After 1900, more attention was paid to fluorosis but, unfortunately, most publications lacked information concerning the fluoride content of the feed and the corresponding clinical symptoms in the affected animals. The first major report on the fluoride content of feedstuffs and its clinical effects on cattle was published in 1934 by PHILLIPS. A very thorough report based on investigations carried out over a period of 8 years can be found in the Fourteenth Report of the German Research Community 1968 (WÖHLBIER et al.).

With increasing industrialization and the infringement of industry upon agricultural areas, the danger of fluorosis in domestic species has increased. This hazard is present by virtue of the fact that in many of these industries fluorine is emitted into the atmosphere. Ceramic and porcelain works, enameling, hydrofluoric acid production, aluminium and phosphate plants, represent only the most important examples of the industries concerned.

For practical purposes, one can consider the increased amounts of fluoride ingested by cattle in Europe as being derived solely from their feedstuff. The fluoride compounds involved are, however, up to now, largely unknown. Plants do not absorb fluoride from the soil, from which one can conclude that its presence in increased amounts in feed is the direct result of contamination through the atmosphere. Observations on plants surrounding an aluminium factory indicate that the concentration of fluoride in plant materials is higher during dry weather, and lower following periods of rainfall. The largest portion of fluoride emitted by an aluminium factory is in form of dust and aerosols (EMPA).

The solubility of the fluoride compounds involved is a decisive factor in the appearance of fluorosis in cattle. It is known that fluorosis appears more rapidly in cattle grazing near hydrofluoric acid producing plants, which emit fluoride in the form of HF, than in those associated with other industries. Through various experiments, it has been proven that the feeding of sodium fluoride will produce fluorosis more rapidly than will the feeding of cryolite. WÖHLBIER et al. have shown that cattle fed cryolite excreted double the amount of fluoride in the feces as compared to cattle fed sodium fluoride. On the other hand, the cattle fed sodium fluoride had relatively higher values for fluoride in the urine. This indicates that NaF is more easily absorbed than cryolite. The accepted level at which fluoride can appear in the feedstuff without economic loss of the animal is 30–40 ppm. The economic usefulness of an animal depends upon the following: age, length of residence in the area, fluoride content of feedstuff, milk production and fertility.

The symptoms are easiest to recognize when the animals have been in a contaminated area since birth or at least prior to the eruption of the permament teeth. When animals ingest an elevated level of fluoride before the

[illegible], brittleness and increased wear. Mottling is characteristic of fluorosis only, if the changes take place symmetrically. Mottling of only one incisor is very often observed in cattle outside a contaminated area. Effects upon the molar arcades are usually first seen when abnormal wearing occurs.

Where the fluoride level in the feed has been as high as 40–60 ppm, exostoses on the extremities, the mandible and ribs may occur. The exostoses on the extremities are first noted at the insertion of tendons, never in articulations. The ribs and the os pedis have a tendency to become brittle and fractures of these bones are common

Clinical symptoms include: stiff gait, decreased appetite, staring haircoat, loss of weight and drop in milk production. A greatly diminished intake of food is observed especially after the molar teeth have undergone abnormal wear As long as the food intake remains normal, fertility and milk production are not affected.

In the presence of fluoride concentrations in excess of 60 ppm, clinical symptoms may develop within one to two years (Table 1). At this time, the animal must be, for economic reasons, slaughtered. If the animals enter a contaminated area for the first time after the eruption of the permanent teeth, no dental changes will occur even in the presence of extremely high fluoride intake Pathologically, the changes are limited to exostoses and fractures

Table 1. Effects of ingested fluorine on dairy cattle. Animals fed various levels of sodium fluoride from 4 months to 7½ years of age. Consolidated data correlating amount of fluorine in the feed with age, amount of fluorine in the tissues, lesions and symptoms (from SCHMIDT, H., et al.).

Average	*Age years*	*Normal conditions*	*Chronic fluorosis*				*Acute fluorosis*
			No adverse effects	*Borderline*	*Moderate*	*Severe*	
F in feed (ppm)	2	up to 15	15–30	30–40	40–60	60–109	over 250
	4	up to 15	15–30	30–40	40–60	60–109	over 250
	6	up to 15	15–30	30–40	40–60	60–109	over 250
Teeth classification (incisors)	2	0–1	0–2	2–3	3–4	4–5	Characterized by: Rapid onset – Reduced food intake – High F content in urine – Loss of body weight – Drop in milk production – Stiffness – Weakness – Even death
	4	0–1	0–2	2–3	3–4	4–5	
	6	0–1	0–2	2–3	3–4	4–5	
Teeth classification* (molars)	2	0–1	0–1	0–1	0–1	0–3	
	4	0–1	0–1	0–1	1–2	1–4	
	6	0–1	0–1	1–2	1–3	1–5	
F in bone (ppm)	2	401–714	714–1605	1605–2130	2130–3027	3027–4206	
	4	706–1138	1138–2379	2379–3138	3138–4504	4504–6620	
	6	653–1221	1221–2794	2794–3788	3788–5622	5622–8676	
F in urine (ppm)	2	2.27–3.78	3.78–8.04	8.04–10.54	10.54–14.71	14.71–19.86	
	4	3.54–5.3	5.3–10.32	10.32–13.31	13.31–18.49	18.49–25.63	
	6	3.51–6.03	6.03–11.29	11.29–14.78	14.78–20.96	20.96–30.09	
F in milk (ppm)	2	up to 0.12	up to 0.12	0.08–0.15	0.15–0.25	0.15 and above	
	4	up to 0.12	*up to 0.12*	*0.08–0.15*	*0.15–0.25*	0.15 and above	
	6	up to 0.12	up to 0.12	0.08–0.15	0.15–0.25	0.15 and above	
F in blood (ppm)	2	up to 0.30	up to 0.30	0.15–0.40	0.30–0.50	0.50 and above	
	4	up to 0.30	up to 0.30	0.15–0.40	0.30–0.50	0.50 and above	
	6	up to 0.30	up to 0.30	0.15–0.40	0.30–0.50	0.50 and above	
F in soft tissue (ppm)	2	up to 1.20	up to 1.20	up to 1.20	up to 1.20	up to 1.20	
	4	up to 1.20	up to 1.20	up to 1.20	up to 1.20	up to 1.20	
	6	up to 1.20	up to 1.20	up to 1.20	up to 1.20	up to 1.20	
Periosteal hyperostosis**	2	0	0–1	0–1	0–2	0–3	
	4	0	0–1	0–1	0–3	0–4	
	6	0	0–1	0–2	0–4	0–5	
Secondary changes may occur***	all	absent	absent	absent	present	present	

* Average classification of full mouth:
Incisor teeth classification:
(Degree of fluoride effects)
0–normal, 1–questionable, 2–slight, 3–mild, 4–marked, 5–excessive
Molar teeth classification:
(Degree of wear)
0–normal, 1–questionable, 2–slight, 3–mild, 4–marked, 5–excessive

** Periosteal hyperostosis:
0–normal, 1–questionable, 2–slight, 3–mild, 4–marked, 5–escessive

*** Stiffness and lameness – Loss of body weight – Reduced feed intake – Rough hair coat – Unpliable skin – Drop in milk production

These data are based on controlled experiments, but can be correlated with numerous field cases that have been extensively studied and evaluated.

In our own observations, we observed no effect upon blood clotting, blood sugar, SGPT, or SGOT. The only change seen was an elevated alkaline phosphatase in animals exhibiting marked exostoses. This would indicate that bone metabolism is activated under the effect of increased fluoride intake. The test for fluoride in the urine has a certain diagnostic value. When the value of fluoride in urine exceeds 10 ppm, the intake is probably sufficient to cause damage. When this has occured there is no specific treatment, the only possible therapy being to move the affected animals to a non-contaminated area.

Attempts have been made to bind part of the fluoride into insoluble compounds by adding so-called alleviators to the feed. For this purpose, aluminum sulfate, aluminum chloride and aluminum acetate have been used. FLATLA determined in his experiments with sheep that through the daily administration of 25 mg aluminum acetate per kg body weight, the fluoride content of the skeleton could be reduced by 50%. With higher doses, however, disturbances in growth appear. The high cost of this feed supplement for domestic animals makes its use prohibitive. It is therefore impossible, even with alleviators, to prevent the appearance of fluorosis permanently. Thus, the only effective and practical method of preventing fluorosis in animals is the purification of gaseous wastes from factories.

Investigations conducted by the Swiss Federal Materials Testing Station in an aluminum factory have demonstrated that the effective removal of 93% of the fluoride in dust and aerosols and 99% of the fluoride in gases is possible The installation costs however are high Nevertheless, industrial concerns must be coerced into using all available methods for the further prevention of air pollution

REFERENCES

ALTHER, E. W.: Chemisch-biologische Untersuchungen zur Fluorose des Rindes. Diss. Landw. Hochschule Hohenheim, Stuttgart, Februar 1961.

EMPA: Eidgenössische Materialprüfungs- und Versuchsanstalt für Industrie, Bauwesen und Gewerbe. Emission von Luftfremdstoffen. Bericht Nr. 39000 (1968).

FLATLA, J. L., quoted after J. R. MARIER: Fluoride research. Science *159*, 1494 (1968).

PHILLIPS, P. H., HART, E. B., and BOHSTEDT, G.: Chronic toxicosis in dairy cows due to the ingestion of fluorine. Agr. Exp. Stat. of the University Wisconsin, Res. Bull. 123 (1934).

SCHMIDT, H., HARRIS, W. F., and SHUPE, J. L.: A report answering specific questions with an evaluation of findings for fluorosis of animals in the region of Nothern Switzerland. Schweiz. Arch. Thkd. *110*, 109 (1968).

WÖHLBIER, W., OELSCHLÄGER, W., GRONBACH, G., und GIESSLER, H.: Die Resorption von Fluor durch Ochsen aus Erde und Flugstaub einer Aluminiumhütte. 14. Forschungsbericht der Deutschen Forschungsgemeinschaft, 114–125 (1968).

Fluoride in the Treatment of Bone Disease

The action of fluoride on diffuse bone atrophies

M. THIÉBAUD, R. ZENDER, B. COURVOISIER, C. A. BAUD and C. JACOT

The action of fluoride was studied on 13 patients (12 females and 1 male) between the age of 49 to 86 years, suffering from bone atrophy. 12 patients had diffuse osteoporosis of senile, post climacteric or idiopathic type and the last patient atrophic PAGET's disease. Fluoride was given in enteric coated tablets of sodium fluoride*, containing 10 mg of fluoride ion. 7 patients received 30 mg of fluoride ion daily during 200 days, 6 received 30 to 70 mg daily during 140 to 300 days.

This following study deals with the biological, histological, radiological and clinical aspects of fluoride treatment including alterations of tissues other than bone.

1. METHODS

Ca: titration with EDTA in the presence of calceine as indicator; P: phosphomolybdate reaction; alkaline phosphatase: substrate is p-nitrophenyl-phosphate; hydroxyproline: urine acid hydrolysis—oxydation to pyrrole—reaction with p-aminobenzaldehyde; F in urine: microdiffusion method of BÄUMLER and GLINZ (1); F in bone: dosage made with the specific electrode according to the technique of RICHARDSON and MCCANN (2).

2. BIOLOGICAL STUDIES

Bone metabolism was checked periodically, every two weeks on an average, by the following parameters: blood calcium, phosphate and alkaline phosphatase and urinary calcium and hydroxyproline. The latter two were determined also before and after the treatment on hospitalized patients receiving a low calcium and collagen diet.

Results: Blood calcium and phosphate levels did not change significantly. After approximately three months of treatment and with a normal diet,

* We wish to thank Zyma S.A., Nyon, for providing the tablets of NaF.

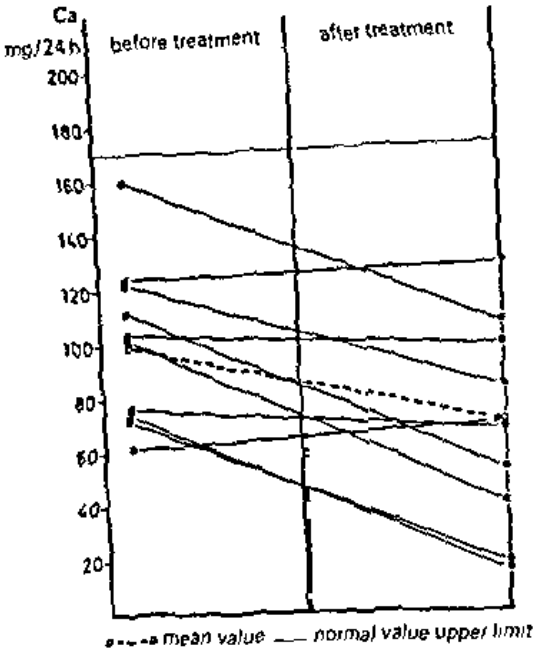


Fig. 1. Urine calcium before and after treatment with NaF.

calciuria decreased slightly but not significantly. However, the urinary calcium of 10 patients receiving a standard low calcium diet showed an average decrease of 33% after treatment in comparison to their calcium excretion before it. According to variance analysis this decrease is significant (Fig. 1). There is no relationship between the amount of fluoride given and the degree of the decrease in urinary calcium. The mean calciuria of each patient remains within normal values, *i.e.* below 170 mg/d.

The organic phase of bone was studied by hydroxyprolinuria analysis. Normal adults given a low collagen diet eliminate less than 40 mg of this amino acid daily. The hydroxyprolinuria of 10 patients showed an average increase of 47% after treatment compared to their hydroxyprolinuria before it (Fig. 2). Considered one by one, these variations are less significant than those of calciuria, the 10 patients studied forming a disparate group. 6 patients presented a definite increase, 3 a slight increase and 1 a decrease of hydroxyprolinuria. Variance analysis showed an interaction significantly higher ($P < 0.001$) than the variability in the patients before and after treatment. Under these conditions the difference, tested between general mean values before and after treatment, with reference to interaction is not significant ($P > 0.05$). However, WILCOXON's test for coupled differences of mean values per subject gives $P = 0.05$, the statistical limit. There

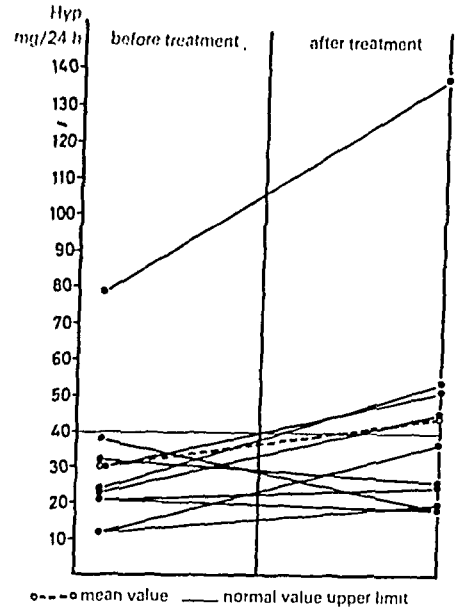


Fig. 2. Urine hydroxyproline before and after treatment with NaF.

is no relationship between the amount of fluoride given and the variations of hydroxyprolinuria.

The increase of hydroxyprolinuria, though not significant, is still interesting, as this amino acid is known to be eliminated in excess in urine during collagen degradation as well as synthesis.

Osteoblastic activity was checked periodically by determination of alkaline phosphatase, which increased definitely and significantly (Fig. 3). The patients presented no liver diseases. Regression analysis of 1' cases during the first 32 weeks of treatment showed a significant and positive relationship between the length of treatment and blood alkaline phosphatase levels. Although dispersion of values around the regression line is considerable, linearity is observed ($P = 0.005$) and the significance test is clearly positive ($0.01 > P > 0.001$).

There is no interrelationship between the variations of calciuria and hydroxyprolinuria. Alkaline phosphatase levels increased after a few weeks treatment, hydroxyprolinuria rose only after several months.

In short, we observed a definite decrease of calciuria, an increase of hydroxyprolinuria and blood alkaline phosphatase levels, suggesting a modification of the balance between resorption and apposition in favour of the latter.

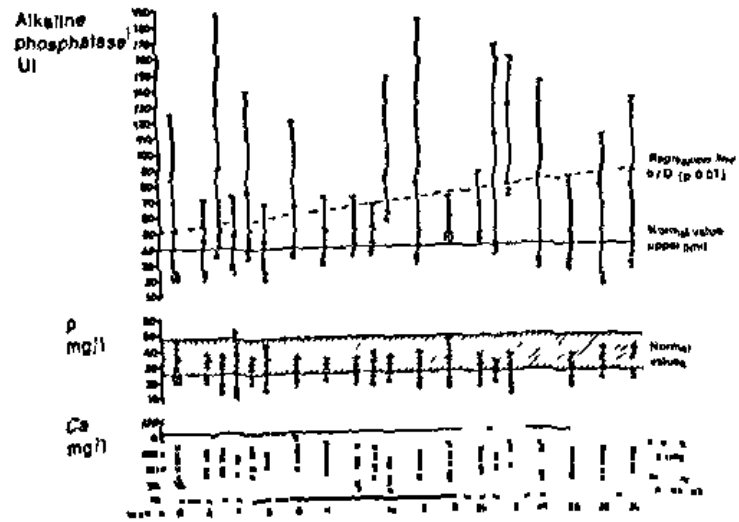


Fig 3. Plasma alkaline phosphatase during treatment with NaF.

3. FLUORIDE DOSAGES

Urinary fluoride excretion of 13 patients was determined during the treatment The daily values obtained were 12 to 30 mg/24 h, representing 30% to 60% with a mean value of 44% of ingested fluoride. Thus, an average of 56% is retained by the patient. Excretion is constant and there is no difference between fluoride retention at the beginning or the end of treatment. The per cent of fluoride ingested that is found in urine does not decrease with the dose Thus patients receiving 60 mg of fluoride retain twice as much as those receiving 30 mg The amount of fluoride in bone biopsies has been determined in 8 cases (Table 1).

Table 1 Fluoride in bone of 8 treated patients

No	*F treatment dosis/day*	*length (months)*	*F % in bone (ashes)*
1	60 mg	6	0.3020
2	70 mg	3	0 1720
6	60 mg	7	0 3550
9	30 mg	6	0 0987
10	30 mg	7	0 3047
12	30 mg	5	0 3010
13	30 mg	6	0 2863
14	30 mg	8	0 2842

It was possible to study the bone specimen of case 6 by X-ray diffraction. The unit cell of apatite was measured; the length of a is shorter than that

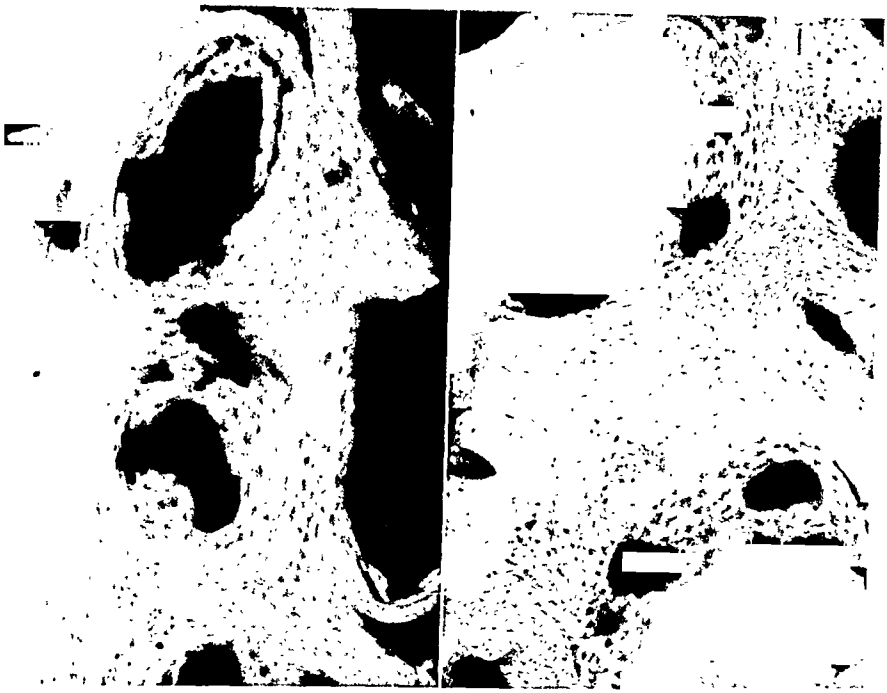


Fig. 4. Fig. 5.

Microradiographs of bone biopsies after treatment showing trabeculae surrounded by mottled bone (× 55)

of the controls, proving that fluoride is incorporated in the crystalline lattice of bone mineral.

4. HISTOLOGY AND MICRORADIOGRAPHY

7 patients underwent a bone biopsy of the iliac at the end of the treatment, 2 patients had biopsies before and after the fluoride treatment. These biopsies were examined by conventional histological methods and by microradiography. They showed typical alterations of osteofluorosis. These alterations can be observed both in compact and cancellous bone. The specific morphological entity, the mottled bone, can be easily identified by microscopy of the microradiographs (Fig. 4 and 5). Mottled bone differs from normal bone in that the former has, 1. a greater number of osteocytes which are irregularly scattered; 2. larger lacunae which may join together and 3. thin, X-ray transparent lines representing apposition defects. Trabeculae of pre-existing bone, surrounded by mottled bone with these typical features, were found in the biopsies, the two structures being clearly separated. The lacunae

of preexisting bone were not enlarged, thus proving that fluoride acted only on osteoblasts and bone formed during the treatment. Intercellular substance of mottled bone did not seem less mineralized than that of adjacent normal bone, but its distribution was more irregular. No conclusion can be drawn from this solely qualitative study as to wether there is an increase in bone mass as a result of fluoride treatment.

5. X-RAY EXAMINATIONS

Comparative study of vertebral column and pelvis X-rays before and after treatment showed no change.

6. CLINICAL RESULTS

8 patients claimed that they felt definitely less bone pain after a few months of treatment. One year after the end of the treatment, 5 of the patients still felt improved. The remaining patients felt either a temporary relief from the treatment or none at all

7 SECONDARY EFFECTS

Fluoride had no side effects except on 2 patients who complained of slight muscular pain in the lower limbs Periodical blood counts, blood sedimentation rates, renal function tests and urinalyses showed no change except leucocyturia without albumin in some elderly patients One case with reduced renal function showed further impairment, which was reversible.

SUMMARY

The action of fluoride was studied in 13 patients between the age of 49 to 86 years 12 with osteoporosis and 1 with PAGET's disease of atrophic type. Sodium fluoride was given daily in doses of 30 to 60 mg of fluoride in ion during 140 to 300 days Clinically, this treatment relieved pain subjectively in 8 patients without modifying X-ray findings. Biological tests showed a decrease in calciuria, an increase in hydroxyprolinuria and in blood alkaline

cancellous bone with typical structural alterations ("mottled bone"), which consists increase of osteocytes located in large lacunae and apposition

defects. Urinary fluoride dosages showed that about 50% of fluoride given is retained by the patient; the bone contained an average of 3‰ of fluoride. The enteric coated tablets of sodium fluoride were well tolerated without any secondary or toxic effects. Considering that the perspective required to appraise the effect of fluoride is lacking and that the newly formed bone induced by this substance is of uncertain quality, the authors feel that treatment with fluoride is still in an experimental stage. The present observations are not to be taken as an indication that fluoride should be used in the treatment of osteopathies of atrophic type in general medicine.

LITERATURE

1 Bäumler, P. J., und Glinz, H.: Bestimmung von Fluoridionen im Mikrogrammbereich. Mitt. Lebensmittel Hyg. *55*, 250 (1964).
2 Richardson, B., and McCann, H. G.: The determination of micro amounts of fluoride by means of fluoride electrode. IADR abstr. *45*, 77 (1967).

Fluoride in osteoporosis

F.W. Reutter, R. Siebenmann and M. Pajarola

Stimulated by Rohlm's description of skeletal fluorosis in cryolite-workers (8) and by the first reports of Rich on therapeutic fluorosis in patients with various forms of bone disease (6, 7) we initiated treatment of severe forms of osteoporosis with fluoride four years ago. First results were reported in 1965 (5). Today, clinical, biochemical and bone biopsy observations on 31 patients with osteoporosis can be reported

PATIENTS AND METHODS

All patients were seen at the Medical department of the Kantonsspital St Gallen Some of the patients were hospitalised during the entire period of treatment, whereas others only for the initial study and control examinations, being followed in between either by their private physician or as outpatients 26 patients had idiopathic osteoporosis and 3 patients rheumatoid arthritis with severe osteoporosis, 2 of whome were on maintenance corticosteroid therapy In two cases osteoporosis was due to complete immobilization because of neurological disease All patients were extensively studied to rule out other possible causes of osteoporosis such as malabsorption, hyperparathyroidism or other endocrine abnormalities.

Fluoride was administered as sodium fluoride in uncoated tablets in a dose of 37–100 mg of fluoride ion per day for periods ranging from 21 days to over three years In some cases additional therapy included vitamin D and/or calcium

Serum calcium, phosphorus, alkaline phosphatase (Bessey) and tartrate-formalin not inhibited acid phosphatase (9) were determined before and at various intervals during treatment in all patients. X-rays of the skeleton were done before and at various times during treatment. Calciuria was measured under low calcium diet (150 mg calcium per day) and calcium infusion studies performed according to Haas in nearly two thirds of the cases (2).

Iliac crest (84 biopsies) and rib (5 biopsies) biopsies were performed in all 31 patients before initiation of fluoride therapy and repeated at various

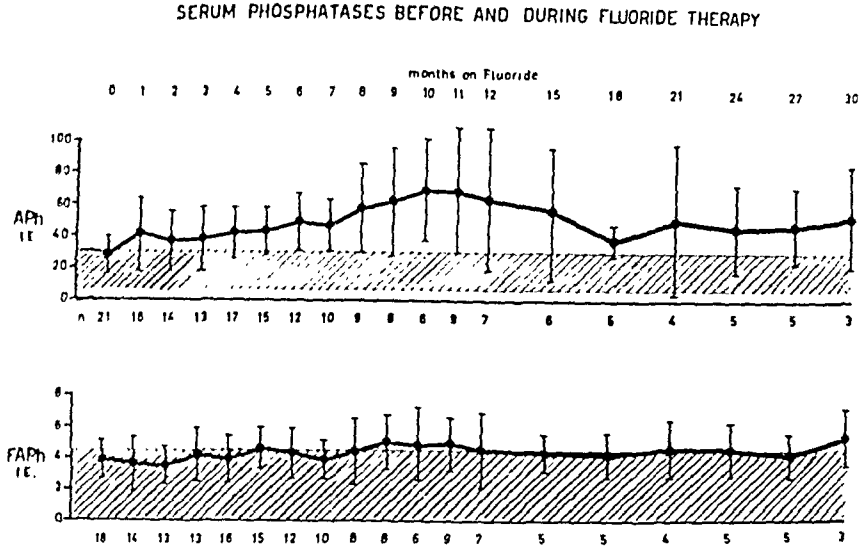


Fig. 1. Mean values and standard deviation of serum alkaline phosphatase (APh) and by formalin and tartrate not inhibited acid phosphatase (FAPh), before and during fluoride therapy. n = number of cases examined.
Increase of APh is of statistical significance for months 1 to 11 ($p < 0.001$–0.05) lacking significance there after. Increase of FTAPh is small and of statistical significance only in months 5 and 9 ($p < 0.05$).

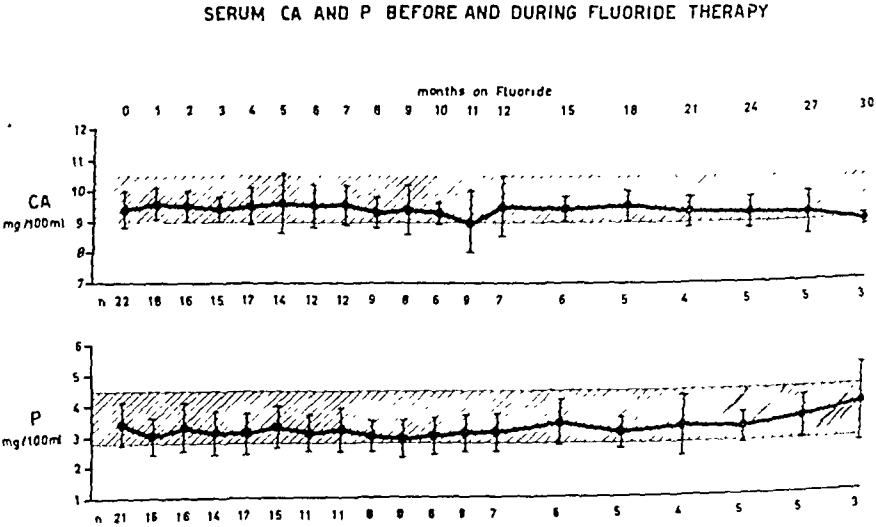


Fig. 2. Mean values and standard deviation of serum calcium and phosphorus before and during fluoride therapy. There is no significant change on fluoride. n = number of patients examined.

intervals. In 12 patients one biopsy was performed while in 19 patients two to five biopsies were done after periods ranging from 28 days up to 950 days of mostly uninterrupted fluoride treatment (4).

Autopsies were performed in 6 patients who died during treatment for reasons not related to fluoride therapy. The skeleton was examined grossly, after maceration as well as histologically (skull, ribs, vertebrae, iliac crest and femur). Biopsies and autopsy material were examined in most cases after decalcification with routine stains, in a few cases also with undecalcified sections stained with ROMANOWSY, FUCHSIN and MALLORY trichrome (kindly prepared for us by Prof. E. UEHLINGER, Zurich). The parathyroid glands were examined in two cases

Pretreatment biopsies confirmed the clinical diagnosis of idiopathic senile osteoporosis in 28 cases, and simple inactivity-osteoporosis, possibly enhanced by corticosteroid treatment, in 3 patients. No evidence of osteomalacia, renal osteopathy or hyperparathyroidism was found.

Statistical significance was determined by t-test.

RESULTS

Biochemical studies

Mean value and standard deviation of serum calcium, phosphorus and phosphatases of 22 patients given fluoride for at least three months are shown in Fig 1 and 2 Fluoride caused an increase in alkaline phosphatase, being significant after one month and persisting for the first year. Thereafter increases in alkaline phosphatase became less pronounced and statistically insignificant The increase in alkaline phosphatase was dose dependent and never observed at a fluoride dose below 37 mg per day. Continuing the same dose of fluoride elevated alkaline phosphatase returned to normal after 1 to $1\frac{1}{2}$ years of treatment in two cases

A small increase in tartrate-formalin not inhibited acid phosphatase did not prove to be significant. This acid phosphatase is a good index for the activity of bone osteoclasts in the absence of liver disease. It is determined by simultanous inhibition of the erythrocyte fraction by tartrate and of the prostate fraction by formalin (9)

No significant change in serum calcium or phosphorus was noticed during fluoride administration (Fig 2).

Urinary calcium excretion was determined in 16 patients Calciuria decreased significantly in all ($p < 0.01$). This reduction was noted one to three months after onset of fluoride therapy and persisted during the entire period of observation lasting up to 18 months (Fig 3).

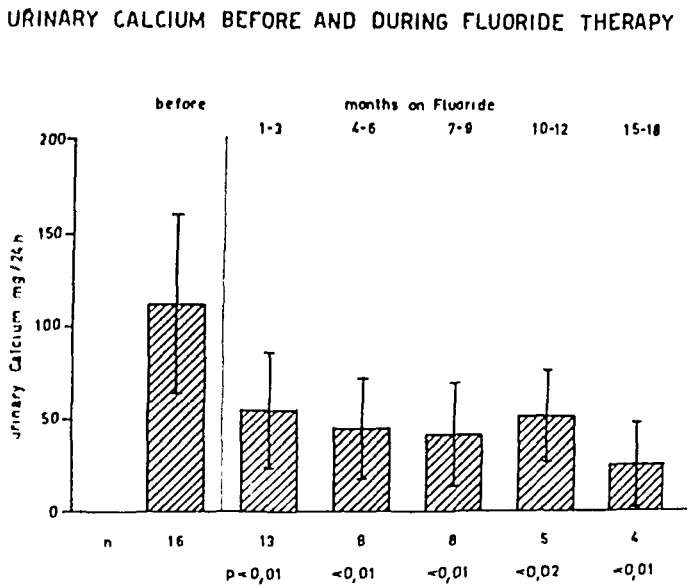


Fig. 3. Mean urinary calcium excretion and standard deviation before and on fluoride. Calciuria decreases significantly on fluoride. n = number of patients examined.

Calcium retention after an intravenous calcium load was already slightly elevated in 11 of 16 patients in the control period. On fluoride, mean calcium retention increased significantly over mean control values during the first nine months of treatment, lacking statistical significance thereafter (Fig. 4).

Radiological changes

There was no change on X-ray films of the spine and pelvis during the first six months on fluoride. Coarse and thickend trabeculae were noticed in approximatively 70% of treated patients after nine to twelve months. Formation of multiple exostoses occured in a 78 year old female after 14 months on fluoride (total dose 29.1 gm). On X-ray, soft tissue calcifications were not seen in any cases. Blurred bone structure and merging of trabeculae resulting in a diffuse structureless bone shadow with increased density of the spine, pelvis and ribs was noticed in a 49 year old female after one year of treatment. The total dose of fluoride in this patient, the youngest one of our series, was 27.2 gm. These X-ray findings correlate with the second phase of osteosklerosis according to ROHOLM (1). Except for a slight decrease in glomerular filtration (average endogenious creatinine-clearance 60 ml/minute) possibly causing higher fluoride retention no explanation for this unique accelerated induction of radiological fluorosis can be given.

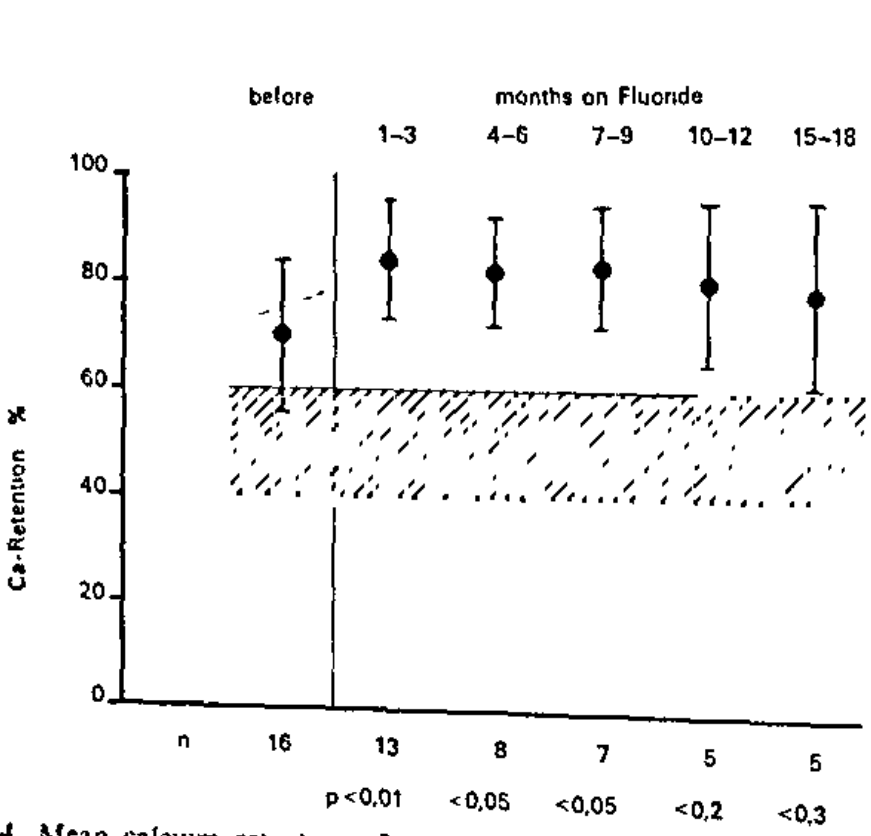


Fig 4 Mean calcium retention after i v calcium load before and on fluoride. Increase of calcium retention is of statistical significance during the first 9 months of treatment

Clinical findings

26 of 31 patients treated with fluoride for more than three months had a decrease of bone pain In 5 patients complete relief of skeletal pain led to marked increase in mobility No improvement was noticed in 5 cases. Bone fractures after minimal trauma occured in 2 patients after several months on fluoride No precise measurement on further vertebral collapse was derived from X-rays but gross progression was rarely observed.

Histological changes

Histologically, slight changes were observed already after 23 days with a total dose of only 1 1 g of fluoride. Clear cut fluorosis, however, was usually found regularly only after three to four months of treatment Macroscopically visible bone changes at autopsy were found only after a years treatment or more

Our biopsy and autopsy findings may be summarized as follows: The therapeutic administration of low doses of fluoride leads to a dose-dependent

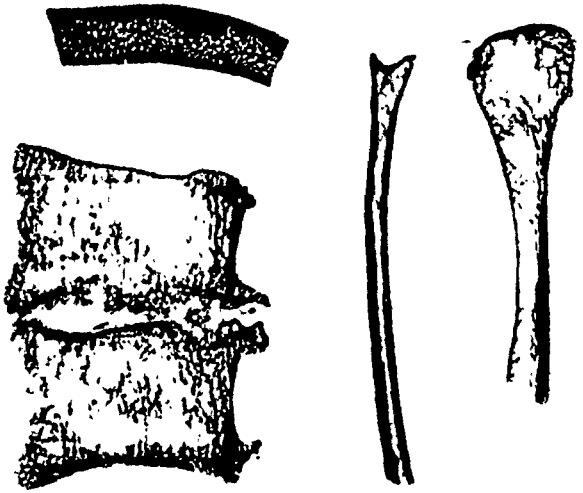


Fig. 5. X-rays of macerated bone sections of a 83 year old female, treated with fluoride for three years. New bone formation resulting in coarse, irregular trabeculae is most pronounced in vertebrae, skull, iliac crest (outer right) and rib.

apposition of a characteristic bone material, which corresponds in all respects to that described earlier in toxic fluorosis (1, 3, 8). This material, which we call fluoride-bone, is richer in osteocytes than normal bone. Its matrix is collagenous, irregularly fibrous at the beginning, but later, i.e. in the deeper layers of apposition, it also becomes lamellar. The material is definitely insufficiently mineralized at the beginning. After several months, however, normal mineralization without other therapy such as calcium- or vitamin D-administration, occurs. Always there remains, however, an inner young layer of osteoid. Mineralization thus seems to be delayed. A characteristic basophilic motteling of the matrix was invariably seen after prolonged treatment.

Apposition of fluoride-bone is irregular from the beginning, but this irregularity is accentuated after longer duration of treatment. Some areas of the bone surface seem not to have been covered at all by fluoride-bone at any time. Periosteal and endosteal deposits of fluoride-bone are found from the beginning.

The periosteal deposits occasionally merge with calcifications and ossifications of tendon-tissue, but these periosteal appositions never became pronounced even after several years of treatment. They were always confined to the immediate bone surface. Thus macroscopically, even in the case treated

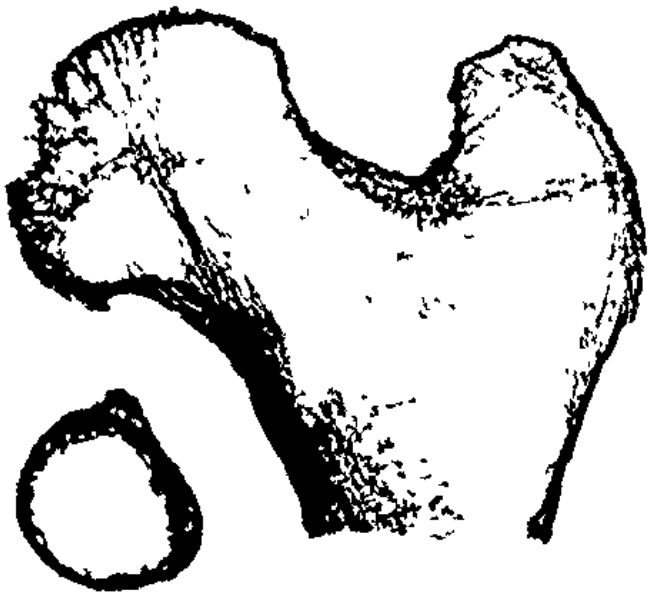


Fig 6 X-rays of macerated sections of femur of same patient, showing irregular new bone formation resulting in a peculiar hypertrophic atrophy of bone

for years, only a roughening of the bone surface, especially at the insertion of ligaments and tendons, was seen Functionally impairing osteophytes never occured

An exception to this rule are to some degree the preexistent osteophytes in spondylotic vertebrae Here an intensification of the osteophytosis was observed Further, in one patient with preexistent bilateral fractures of the femoral neck, one treated by neck prothesis (THOMPSON) and the other by osteosynthesis, luxuriant periosteal fluoride-bone callus was formed at the site of operation

Endosteal deposition of fluoride-bone however, prevailed in every case. This deposition, irregular in extent, coated HAVERSian canals, inner surfaces of compact bone and trabeculae of cancellous bone. The most pronounced appositions occured on the bone trabeculae. The width of the deposited fluoride-bone often greatly exceeded the thickness of the original trabeculum after only a few months of therapy.

On the other hand, cortical and trabecular bone always show from the beginning of treatment an increased bone resorption. Even in the case treated for only three weeks an increase of patchy bone resorption is seen in comparison to the pretreatment biopsy. This resorption is often present in the classic form of fibroclasia with lacunae Rarer and later canaliculi filled with loose fibrous tissue and occasionally with the typical osteoclasts are

observed. Sometimes only lacunae, and an irregular shape of the periosteal or endosteal surface are seen without fibroosteoclasia thus representing remainders of active bone resorption. Sometimes they were newly covered by fluoride-bone. This bone resorption definitely increases with the length of treatment. There are, however, fluctuations or local differences explaining regression of resorption phenomena on later biopsies. Bone resorption is completely irregular. No evidence was found that fluoride-bone formation is the consequence of bone resorption as a repair mechanism, nor is resorption confined to the newly formed fluoride-bone. Resorption involves preexistent bone as well as fluoride-bone, even in its unmineralized form. This is in contrast to true osteoid, which is not attacked by fibroosteoclasia.

The appearance of bone resorption is indistinguishable from the resorption seen in secondary or primary hyperparathyroidism. Considerable fibrosis of bone marrow was seen only in a patchy manner in two biopsies after long treatment. It was not seen in any impressive extent in the five autopsies, although some cases were treated for several years. The overall result, especially after long term treatment, is a patchy new bone formation, without discernible distribution along mechanically stressed structures. In between there is a persistent or even enhanced porosis. The result is sometimes, especially in the ribs, in the vertebrae and in the femur metaphysis a hypertrophic atrophy (Fig. 5, 6). Quantitative histological studies on bone structure and remodeling are reported separately (R. K. SCHENK, et al., p. 153).

Autopsy findings

Examination of the internal organs of six autopsies revealed no pertinent changes. The parathyroid glands, examined in two autopsies after 12 and 21 months of continuous treatment showed mild hyperplasia with replacement of interstitial fat, increase of small chief cells and some small clear cells. Increased, somewhat nodular islets of oxyphil cells were seen in one patient. Both patients had shown no clinical signs of this anatomically visible secondary hyperparathyroidism. Specifically, there was no renal disease which could explain a secondary hyperparathyroidism. Slight nephrocalcinosis of the medullary region was seen in two of the six cases. It did not exceed that commonly found in patients of that age group with senile osteoporosis. No calcifications of soft tissues far from the skeleton or of any internal organs were seen.

CONCLUSION

Treatment with sodium fluoride of patients with osteoporosis leads to an increase of serum alkaline phosphatase and increased formation of a typically structured new bone, so called fluoride-bone. This indicates stimulation of osteoblastic activity by fluoride Decrease of calciuria and increase of calcium retention after a intravenous calcium load are indirect proofs of a positive calcium balance, a mandatory sequal of excessive new bone formation

We think the anatomical effects of a low dose sodium-fluoride treatment are beneficial in idiopathic senile osteoporosis by virtue of the demonstrable formation of fluoride-bone. This bone is certainly insufficiently mineralized at the beginning of its deposition, but mineralizes after some months. The new bone formation is patchy, however, strictly appositional to existing bone and not especially located at the sites of the greatest need for consolidation. On the other hand sodium fluoride administration increases bone resorption and there is some evidence that this is due at least in part to secondary hyperparathyroidism of non renal origin.

These biochemical and histological findings are paralleled by late changes on X-rays as broadened trabeculae and occasional increased bone density. Though difficult to judge, fluoride seems to decrease skeletal pain in a high proportion of treated patients

It seems that treatment should be limited approximately to a year, or until symptoms are relieved. An intermittent treatment or renewed therapy in later phases seems advantageous.

No untoward effects in other organs have been found.

REFERENCES

1 Baylink, D., and Bernstein, D. *The effects of fluoride therapy on metabolic bone disease* Clin Orthop *55*, 51 (1968)

2 Haas, H G Knochenstoffwechsel und Parathyreoidea [illegible] Georg Thieme [illegible]

3 Johnson, [illegible] Fluorine [illegible]

4 Pajarola, H. Die Knochenveränderungen bei Natriumfluoridbehandlung rarefizierender Skeletterkrankungen Dissertation Zürich (1969).

5 Reutter, F W, und Siebenmann, R. Die Wirkung von Natriumfluorid bei metabolischen Knochenerkrankungen Helv. med. Acta 32, 493 (1965).

6 Rich, C, and Ensinck: Effect of sodium fluoride on calcium metabolism of human beings. *Nature (London) 191*, 184 (1961)

7 Rich, C., Ensinck, J., and Ivanovich, P.: The effects of sodium fluoride on calcium metabolism of subjects with metabolic bone diseases. J. clin. Invest. *43*, 545 (1964).
8 Roholm, K.: Fluoride intoxication: A clinical hygienic study. H. K. Lewis, London (1937).
9 Weber, H., and Wegmann, T.: Atlas der klinischen Enzymologie. Georg Thieme, Stuttgart (1967).

Fluoride in osteoporosis

Quantitative histological studies on bone structure and bone remodelling in serial biopsies of the iliac crest

R. K. SCHENK, W. A. MERZ and F. W. REUTTER

INTRODUCTION

Histological bone changes in patients treated with sodium fluoride have been reported previously by BAYLINK and BERNSTEIN (1967), BERNSTEIN and COHEN (1967), EPKER (1967) and JOWSEY, SCHENK and REUTTER (1968) According to these papers, the most conspicuous findings are a net gain in bone substance, apparently due to an increased bone formation, but accompanied by mineralization defects and slight structural abnormalities of the lamellar bone which has been formed during the period of fluoride administration Quantitative histological investigations however, are rare and confined to a small number of patients and biopsies.

This study deals with structural and turnover changes in serial biopsies, collected from a group of patients suffering from true senile osteoporosis Cases of rarefying bone disease due to other causes have been excluded by thorough clinical and roentgenological examination. For detailed information on case histories, X-ray examination and laboratory findings we refer to REUTTER et al. (p 143).

MATERIAL AND METHODS

The biopsies have been taken from the iliac crest by means of the myelotomy device designed by BURKHARDT (1966). They were repeated in intervals of three to six months, using both sides alternately at varying distances from the anterior superior spine. Tetracycline was usually given five days before each biopsy. The specimens were fixed in 40% alcohol, dehydrated and block stained in 0.5% fuchsin-alcohol and embedded in methylmethacrylate. 50-70 μ thick ground sections served for microradiographic examination and fluorescence microscopy. Quantitative histological evaluation was car-

ried out on 5 μ thick microtome sections stained by a modified GOLDNER technique (SCHENK 1965, SCHENK, MERZ and MUELLER, in press). This method allows a clear distinction between osteoid and calcified matrix, as well as a reliable differentiation of osteoblasts and osteoclasts, bone marrow and connective tissue cells. As a consequence, evaluation of bone turnover is based not only on surface measurement of osteoid seams and of HOWSHIP's lacunae, but also on surface extent of osteoblast layers and number of osteoclasts. Thus it reflects more accurately the actual state of formation and resorption activity. All the measurements were performed with the help of an integrative eyepiece described by MERZ (1967). For a detailed discussion of these criteria, their normal values and age changes, see SCHENK, MERZ and MUELLER (in press), and MERZ and SCHENK (in press).

The results are compared with standard values calculated from 38 normal autopsy cases of an identical age distribution. A total of 48 biopsies, collected from 16 female patients with senile osteoporosis (mean age 74 years) could be examined. This figure includes 9 control biopsies taken at the beginning of the treatment. The remaining 39 biopsies are distributed over an observation period of as much as three or four years. In some instances, a total of five or six bone samples could be gathered from the same individual. For technical reasons, the intervals between the biopsies could not be fixed in advance. In addition, the daily intake of sodium fluoride, usually 50–75 mg, had to be adapted to the general condition of the patient. When the malacic state became obvious after a few months of treatment, vitamin D_2 was added to the fluoride medication. Subsequently all of the cases included later on in the study had a combined treatment with fluoride and vitamin D_2, whereas the original group received supplementary vitamin D only in later periods of observation.

These inevitable drawbacks of the study protocol are satisfactorially compensated by grouping the biopsy specimens according to Table 1. For each group it indicates, beside the number of biopsies and the months of treatment, the mean duration of treatment in weeks and the average accumulated dosage of sodium fluoride in grams. The mean duration of treatment is subsequently used as reference number in the diagrams illustrating the morphometric results.

Table 1. Duration of treatment, accumulated dosage of sodium fluoride, and distribution of biopsies collected from 16 female patients with senile osteoporosis (mean age: 74 years, total of biopsies: 48)

Months of treatment before biopsy	0	1	2–3	4–7	8–14	15–49
Mean duration of treatment before biopsy in weeks	0	5	10	20	40	85
Accumulated dosage of sodium fluoride in grams (S.D.)	0	2.500 (0.592)	4.500 (1.013)	10.800 (2.667)	18.000 (8.073)	27.100 (12.495)
Number of biopsies examined	9	10	6	8	9	6

RESULTS

Qualitative histological changes in the cancellous bone of the iliac crest under prolonged administration of sodium fluoride are first illustrated by a series of four biopsies obtained from the same female patient. This patient had a combined treatment with fluoride (40 mg NaF/day) and vitamin D. At the beginning of treatment, the cancellous bone consisted of trabecula with small diameters (Fig. 1). Osteoid seams, osteoblasts and osteoclasts are rare. After ten weeks of fluoride application, the structure is almost identical, but an increased number of newly formed osteoid seams became evident, suggesting a stimulation of osteoblastic activity (Fig. 2). This statement is confirmed by a corresponding augmentation of osteoblasts. The third biopsy (Fig. 3) represents the typical alterations observed in the second half year of treatment. The trabecular surface is covered to a great extent by osteoid seams of considerable thickness, resembling an osteomalacic state due to a deficiency in mineralization. At the end of the first year, however, an improvement in mineral density of the newly formed areas became obvious. The final stage after 27 months is illustrated by a last biopsy (Fig. 4). Seven months before this biopsy, the application of both fluoride and vitamin D was discontinued. Histologically, osteoid seams are no longer predominant (Fig. 4a). The microradiograph (Fig. 4b) reveals an overall improvement in the degree of mineralization, though some lack of mineral is still detectable in undermineralized halos around the osteocytes and areas of low density in the appositional layers of the first year.

ried out on 5 μ thick microtome sections stained by a modified GOLDNER technique (SCHENK 1965, SCHENK, MERZ and MUELLER, in press). This method allows a clear distinction between osteoid and calcified matrix, as well as a reliable differentiation of osteoblasts and osteoclasts, bone marrow and connective tissue cells. As a consequence, evaluation of bone turnover is based not only on surface measurement of osteoid seams and of HOWSHIP's lacunae, but also on surface extent of osteoblast layers and number of osteoclasts. Thus it reflects more accurately the actual state of formation and resorption activity. All the measurements were performed with the help of an integrative eyepiece described by MERZ (1967). For a detailed discussion of these criteria, their normal values and age changes, see SCHENK, MERZ and MUELLER (in press), and MERZ and SCHENK (in press).

The results are compared with standard values calculated from 38 normal autopsy cases of an identical age distribution. A total of 48 biopsies, collected from 16 female patients with senile osteoporosis (mean age 74 years) could be examined. This figure includes 9 control biopsies taken at the beginning of the treatment. The remaining 39 biopsies are distributed over an observation period of as much as three or four years. In some instances, a total of five or six bone samples could be gathered from the same individual. For technical reasons, the intervals between the biopsies could not be fixed in advance. In addition, the daily intake of sodium fluoride, usually 50–75 mg, had to be adapted to the general condition of the patient. When the malacic state became obvious after a few months of treatment, vitamin D_2 was added to the fluoride medication. Subsequently all of the cases included later on in the study had a combined treatment with fluoride and vitamin D_2, whereas the original group received supplementary vitamin D only in later periods of observation.

These inevitable drawbacks of the study protocol are satisfactorially compensated by grouping the biopsy specimens according to Table 1. For each group it indicates, beside the number of biopsies and the months of treatment, the mean duration of treatment in weeks and the average accumulated dosage of sodium fluoride in grams. The mean duration of treatment is subsequently used as reference number in the diagrams illustrating the morphometric results.

Table ... tribution ... (mean ... dis- rosis

Months of treatment before biopsy	0	1	2–3	4–7	8–14	15–49
Mean duration of treatment before biopsy in weeks	0	5	10	20	40	85
Accumulated dosage of sodium fluoride in grams (S D)	0	2 500 (0.592)	4 500 (1 013)	10 800 (2 667)	18 000 (8 073)	27 100 (12.495)
Number of biopsies examined	9	10	6	8	9	6

RESULTS

Qualitative histological changes in the cancellous bone of the iliac crest under prolonged administration of sodium fluoride are first illustrated by a series of four biopsies obtained from the same female patient. This patient had a combined treatment with fluoride (40 mg NaF/day) and vitamin D. At the beginning of treatment, the cancellous bone consisted of trabecula with small diameters (Fig 1) Osteoid seams, osteoblasts and osteoclasts are rare After ten weeks of fluoride application, the structure is almost identical, but an increased number of newly formed osteoid seams became evident, suggesting a stimulation of osteoblastic activity (Fig. 2). This statement is confirmed by a corresponding augmentation of osteoblasts. The third biopsy (Fig 3) represents the typical alterations observed in the second half year of treatment The trabecular surface is covered to a great extent by osteoid seams of considerable thickness, resembling an osteomalacic state due to a deficiency in mineralization. At the end of the first year, however, an improvement in mineral density of the newly formed areas became obvious. The final stage after 27 months is illustrated by a last biopsy (Fig. 4). Seven

(Fig 4a). The microradiograph (Fig. 4b) reveals an overall improvement in the degree of mineralization, though some lack of mineral is still detectable in undermineralized halos around the osteocytes and areas of low density in the appositional layers of the first year.

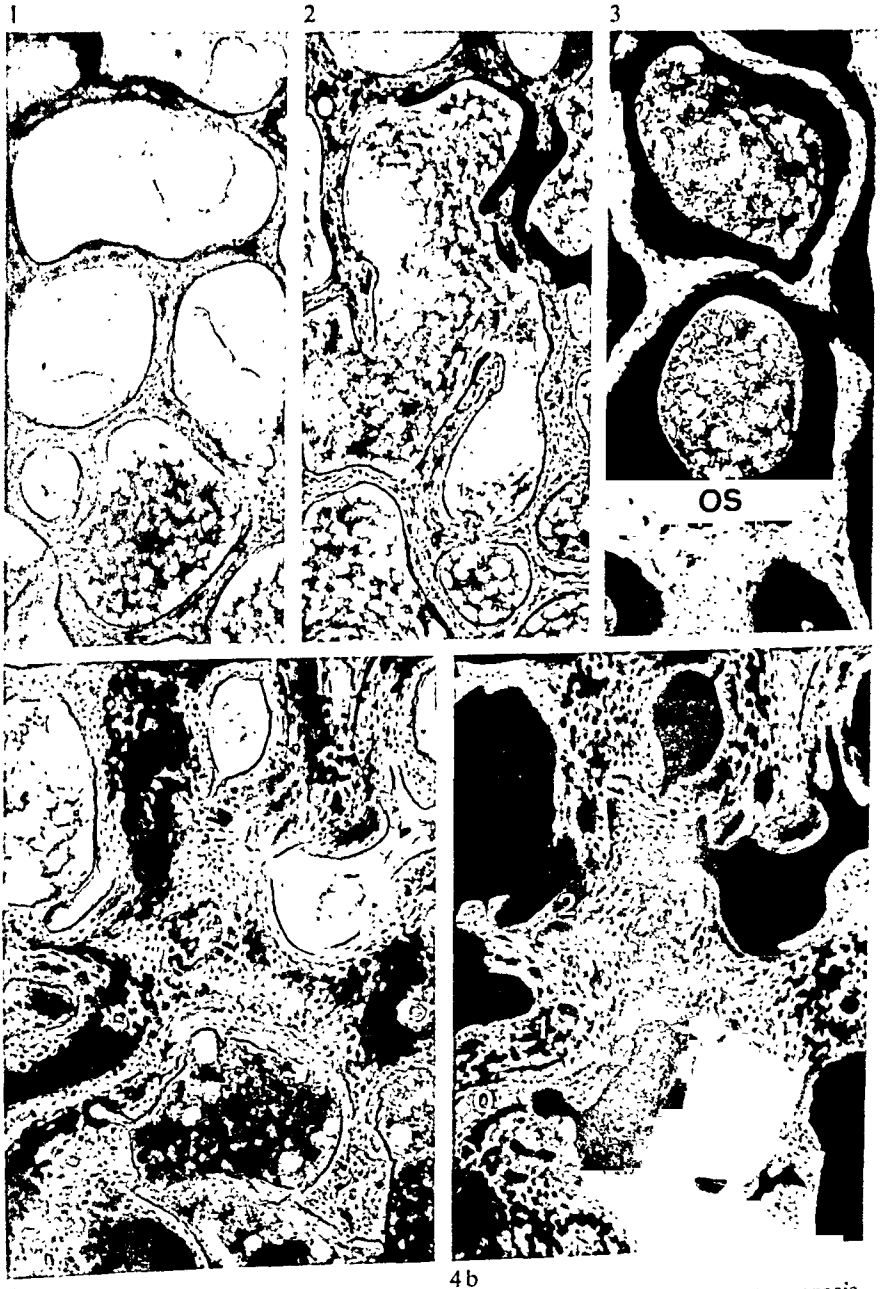


Fig. 1–4. Serial iliac crest biopsies of a female patient with senile osteoporosis, treated with sodium fluoride. Fuchsin-stained, non-decalcified ground sections, 50:1.

Fig. 1. Control biopsy at the beginning of the therapy.

Fig 2. Biopsy after 10 weeks of sodium fluoride, 40 mg/day. Fuchsin-stained osteoid seams appear as dark bands

Fig 3. 7 months after the beginning of treatment, most of the trabecular surface is covered by wide osteoid seams (OS).

Fig 4 Biopsy after 27 months of treatment. a—bright field, b—microradiograph 0 = pre-existing bone. 1 = bone formed during the first year of treatment. 2 = appositional layers formed in the second year Note the striking improvement in osteoid mineralization Fluoride-containing areas of the first year are clearly recognizable by their relatively low mineral density and enlarged osteocyte lacunae. In contrast to this, the recently formed layers have an almost normal appearance (2)

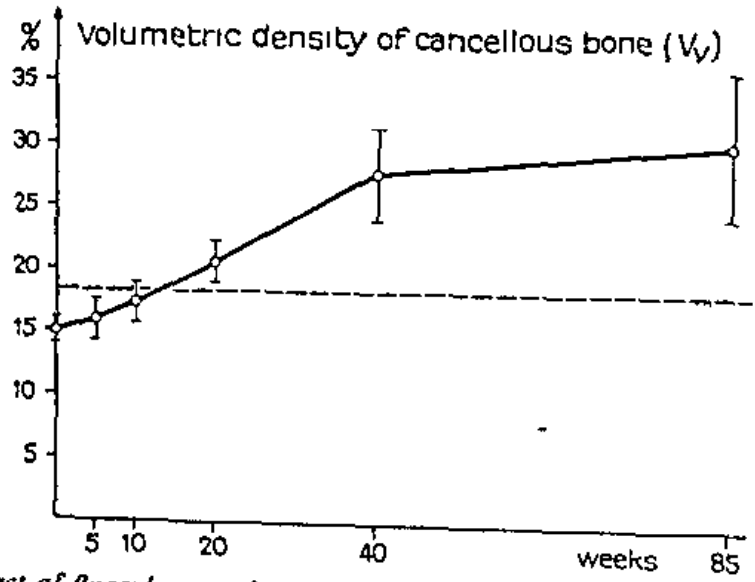


Fig 5 Effect of fluoride on volumetric density = ratio bone to total volume of cancellous bone (bone + bone marrow) This percentage comprises both mineralized and unmineralized matrix

This outline of morphological changes raises a number of questions which may best be explained by means of diagrammatic representations of the results of our quantitative histological measurements. In these diagrams (Fig 5-9), the dark line and brackets indicate mean values and standard error of the groups, whereas the dashed line and the dotted strip mark the average and standard error of normal values for the corresponding age group

Changes in bone structure are suitably evaluated by determination of volumetric density and specific surface of trabecular bone. Definitions of these criteria are given in the legends of the respective graphs. Volumetric density of cancellous bone (Fig 5) is, at the beginning of the therapy, definitely below the normal level. This confirms the presence of osteoporosis in the group of patients selected for this study. [illegible]

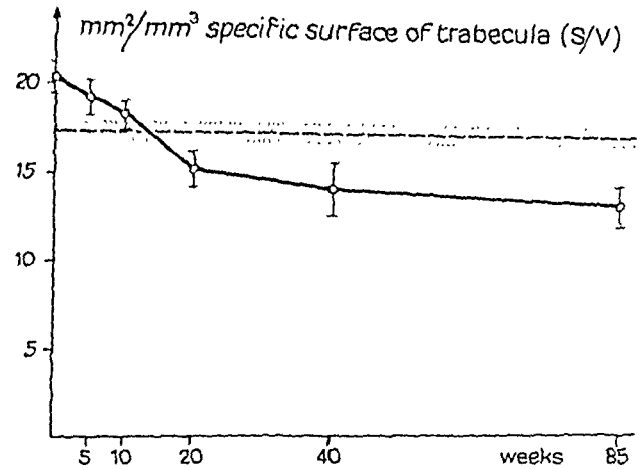


Fig. 6. Effect of fluoride treatment on specific surface of trabecula (trabecular surbone) This value is converse to the mean trabecularface per unit volume of trabecular thickness. High figures indicate a small, low figures a large trabecular diameter.

year, but still present in later periods. Thus, the final value almost doubles the original one. The curve obtained for the specific surface shows an opposite trend (Fig. 6). Specific surface is reciprocal to the mean diameter of the trabecula. The initial high value is characteristic for an atrophic trabecular framework. The constant decline under treatment reflects a coarsening of the trabecula, apparently due to the apposition of new osteoid layers upon the surface of the preexisting scaffold.

Both structural values agree with a net gain in bone substance under the influence of fluoride. This positive skeletal balance may be attributed to an inhibition of bone resorption, a stimulation of bone formation, or a parallel, but unequally large acceleration of both osteoclastic and osteoblastic activity. Among the different parameters used for determination of formation and resorption activities, the osteoclast index (Fig. 7) and the surface extent of osteoblasts (Fig. 8) are especially informative. The number of osteoclasts shows a slight, but not significant tendency towards higher values. The behaviour of the osteoblasts is, on the other hand, highly characteristic. Starting from an initial, very low value, there is a tremendous rise up to a maximum reached after 40 weeks, which increases eventually to 17 times higher than the minimum. In the second year, there is a decline, but our latest group of observations still exhibits a mean value well above the average of the controls.

The amount of osteoid present in the second half year of treatment Fig. 3) can only be partly accounted for by the increase in osteoblastic

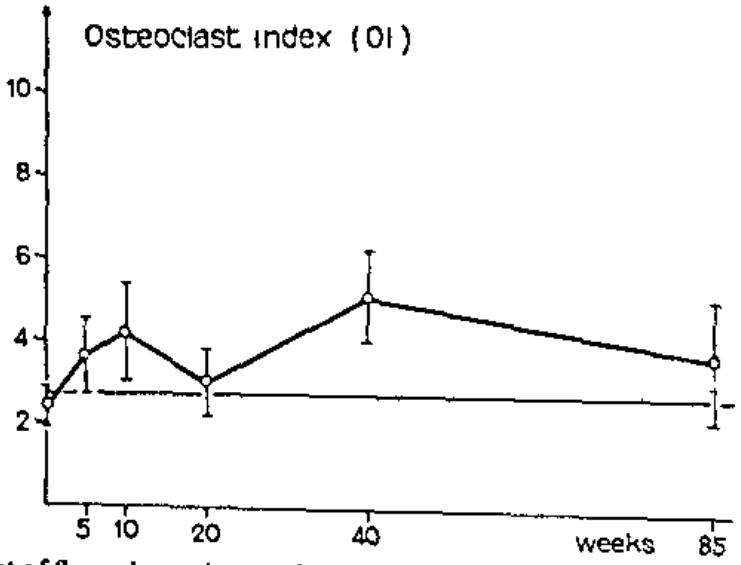


Fig 7 Effect of fluoride on the number of osteoclasts. The osteoclast index indicates the number of osteoclasts per unit trabecular surface.

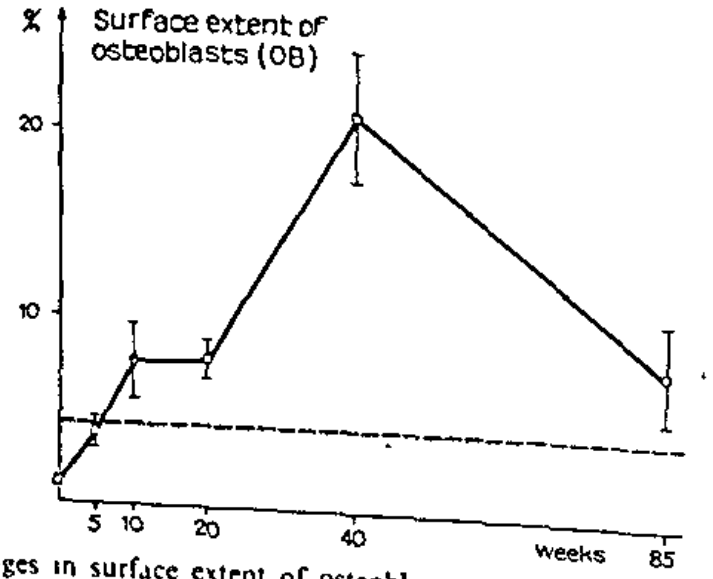


Fig 8 Changes in surface extent of osteoblasts under the influence of fluoride application This value represents the percentage of trabecular surface covered by osteoblasts

activity The presence of extensive, wide osteoid seams already suggests a lack of mineralization. This microscopic feature is verified by measurements of surface extent and mean thickness of osteoid seams The discrepancy between the increased formation of organic bone matrix by osteoblasts

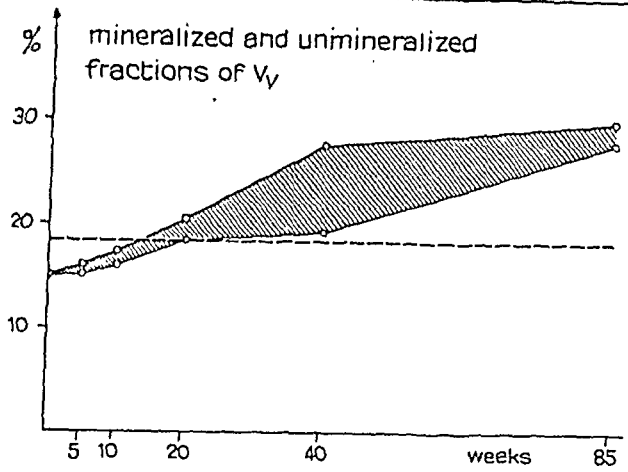
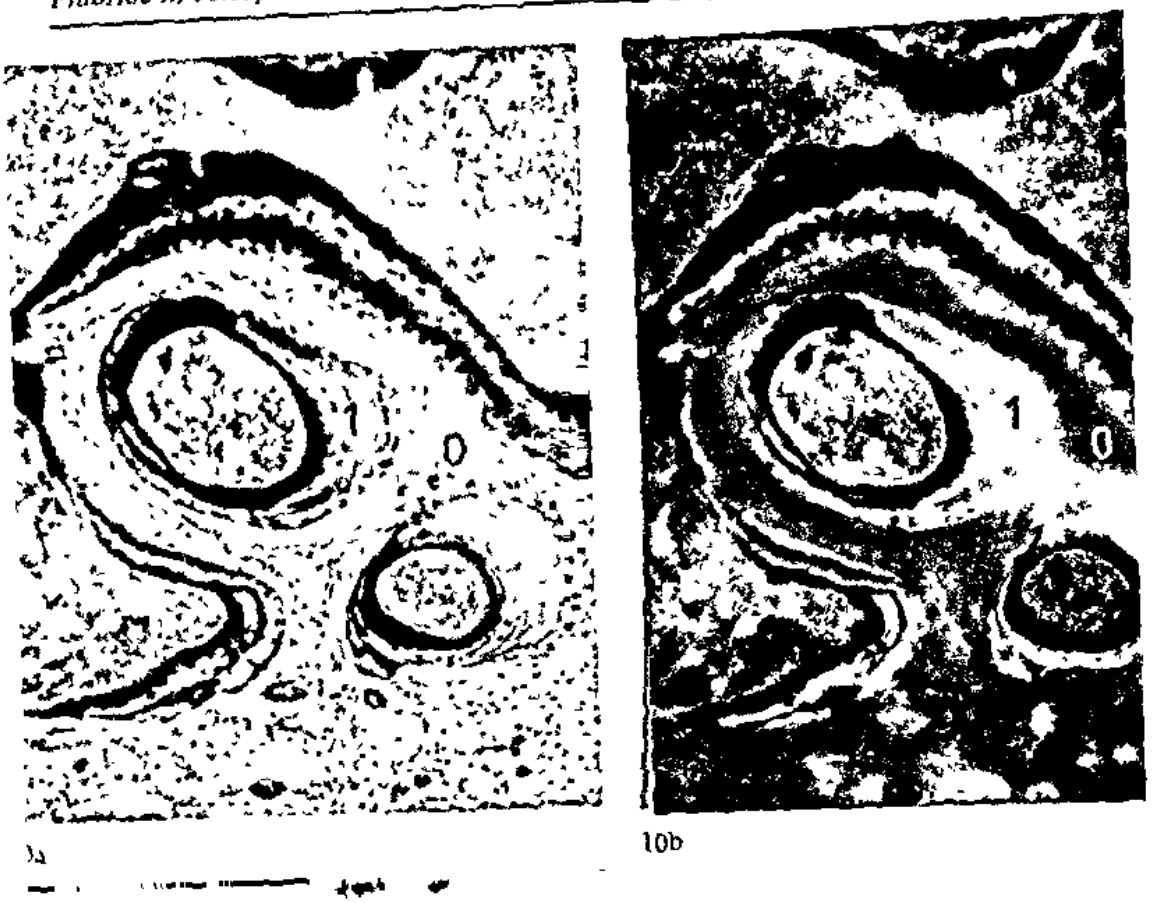


Fig. 9. Effect of fluoride on volumetric density of mineralized and unmineralized fractions of cancellous bone. The upper line represents the total bone substance, the lower line the mineralized part. The hatched area indicates the amount of osteoid present after the respective duration of treatment.

Fig. 9). There is, however, not a complete block of mineralization, as the mineralized fraction of bone also augments progressively, though at a much slower rate. In the second year, the relative amount of osteoid diminishes more and more. The recovery from mineralization impedance is detectable in all 5 patients observed over a period of more than one year. Its histological and microradiographic appearance can best be demonstrated on the basis of a 75 year old woman, which, in addition to iliac crest biopsies, underwent two surgical rib biopsies at the end of the first and second year. From the beginning of treatment, tetracycline was given in regular intervals of five to six weeks. As a consequence of the delay in mineralization, these labels did not appear in the usual form of clearly demarcated fluorescent lines, but rather as diffuse, continuous areas (Fig. 10b). This area represents the appositional bone formed during the first year, its mean width being approximately 110 μ. The preexisting bone in the cortex and trabecula is clearly discernible by its dark green color in the fluorescence micrograph. More than 50% of the trabecular surface is covered by osteoid seams with a mean thickness of 50 μ and a hazy demarcation line. The low mineral content of the tetracycline-labelled area is first indicated by an increased permeability for basic fuchsin, especially prominent in the matrix surrounding the osteocytes (halo volume, FROST, VILLANUEVA and ROTH, 1960). This statement is confirmed by a microradiograph (Fig. 10c), showing poor mineralization of the fluoride-containing bone, uncalcified cement lines and areas of low mineral density around osteocyte lacunae.



10a 10b 10c

Fig 10 Rib biopsy of a 75 year old woman at the end of the first year of fluoride application The periosteal surface of the rib cortex runs parallel to the lower margin of the micrographs Fuchsin-stained ground section, 40 1.

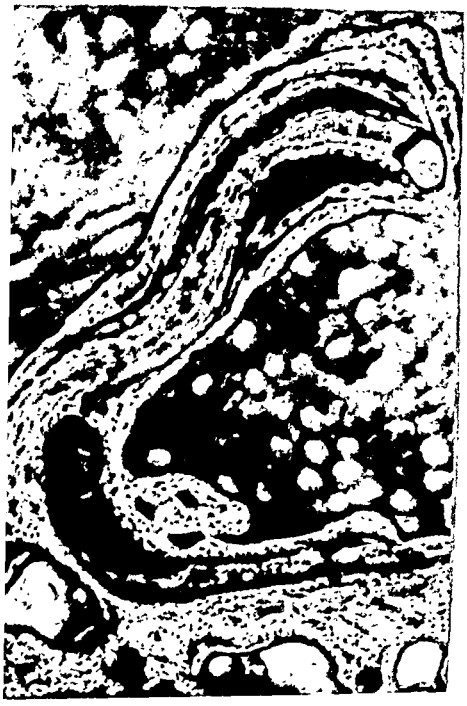
a — bright field

b — fluorescence micrograph

c — microradiograph

0 — preexisting bone

1 = tetracycline-labelled bone, formed during fluoride application



11b

11c

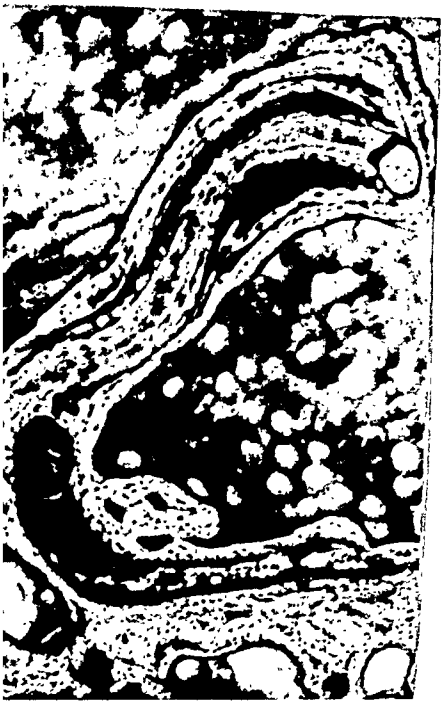
Fig. 11. Rib biopsy of the same patient, one year later (two years after beginning of treatment). 40:1.
a — bright field
b — fluorescence micrograph
c — microradiograph
0 = preexisting bone
1 = tetracycline-labelled bone of the first year,
2 = unlabelled bone formed during the second year

During the second year, the daily intake of sodium fluoride was reduced to 25 mg/day. No further tetracycline was given, in order to obtain a clear histological differentiation between preexisting bone and appositional layers formed during the first and second year (Fig. 11b). In the second year there is still a marked activity of the osteoblasts leading to a further coarsening of the trabecula. In contrast to the first year this newly formed bone has an almost normal appearance and exhibits a definitely higher degree of mineralization. This can be shown both by the uptake of basic fuchsin Fig 11a) and by microradiography (Fig. 11c). In addition, some local) improvement of mineral density in the tetracycline-labelled areas originating from the first year is suggested by the microradiographs.

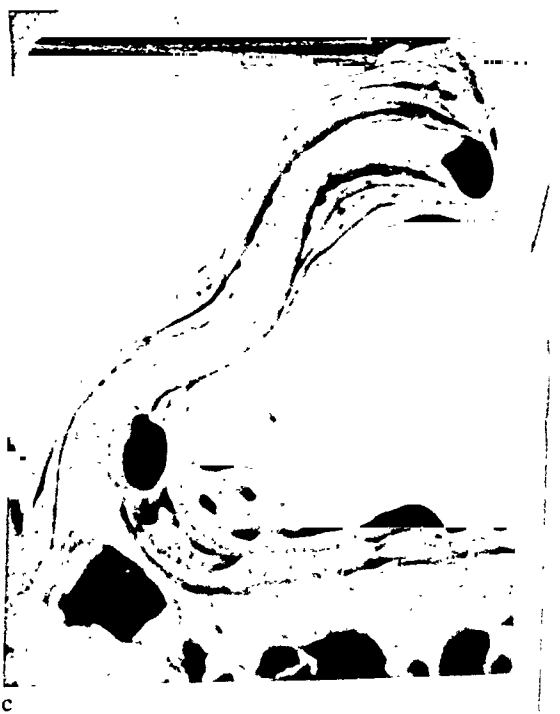
DISCUSSION

The initial control biopsies collected before fluoride application confirm the clinical diagnosis of senile osteoporosis: Volumetric density is definitely below the average of the corresponding normal age group, and high specific surface indicates a reduction of the mean trabecular diameter. This trabecular atrophy is due to a failure in osteoblastic activity, whereby the number of osteoclasts lies within the normal range This finding is contrary to the current view, that negative skeletal balance in osteoporosis is caused by an increased resorption (Jowsey 1967, Sedlin, Frost and Villanueva 1963) It is confirmed, however, by a more extensive morphometric study of bone

reaches and even exceeds the mean value in normal autopsy cases of the same age group. Concurrent reduction of the specific surface demonstrates, that this increase in volumetric density is brought about by a gradual deposition of new, slowly mineralizing osteoid along the surface of the trabecula. The poor mineralization of these appositional layers suggests the presence of osteomalacia, a common finding in various histological investigations dealing with fluoride effects in the skeleton (Bartolucci 1912, Bauer 1945, Brlanger et al 1958). Accumulation of osteoid on the trabecular surface makes a quantitative estimation of bone formation by means of the extent of osteoid seams difficult (Jowsey, Schenk and Reutter 1968). For this reason, our estimation of bone turnover is based directly on measurements of the surface covered by osteoblasts, and as far as resorption is concerned, on osteoclast counts The most conspicuous and constant finding is the tremendous increase in the number of osteoblasts towards the end of the



11b



11c

Fig. 11. Rib biopsy of the same patient, one year later (two years after beginning of treatment). 40:1.
a — bright field
b — fluorescence micrograph
c — microradiograph
0 = preexisting bone
1 = tetracycline-labeiled bone of the first year,
2 = unlabelled bone formed during the second year

During the second year, the daily intake of sodium fluoride was reduced to 25 mg/day. No further tetracycline was given, in order to obtain a clear histological differentiation between preexisting bone and appositional layers formed during the first and second year (Fig. 11b) In the second year there is still a marked activity of the osteoblasts leading to a further coarsening of the trabecula. In contrast to the first year this newly formed bone has an almost normal appearance and exhibits a definitely higher degree of mineralization. This can be shown both by the uptake of basic fuchsin Fig. 11a) and by microradiography (Fig. 11c). In addition, some local) improvement of mineral density in the tetracycline-labelled areas originating from the first year is suggested by the microradiographs

DISCUSSION

The initial control biopsies collected before fluoride application confirm the clinical diagnosis of senile osteoporosis: Volumetric density is definitely below the average of the corresponding normal age group, and high specific surface indicates a reduction of the mean trabecular diameter. This trabe-

... now, that negative skeletal balance in osteoporosis is caused by an increased resorption (JOWSEY 1967, SEDLIN, FROST and VILLANUEVA 1963). It is confirmed, however, by a more extensive morphometric study of bone

augmentation of bone substance, which, after approximately 20 weeks, reaches and even exceeds the mean value in normal autopsy cases of the same age group Concurrent reduction of the specific surface demonstrates, that this increase in volumetric density is brought about by a gradual deposition of new, slowly mineralizing osteoid along the surface of the trabecula. The poor mineralization of these appositional layers suggests the presence of osteomalacia, a common finding in various histological investigations dealing with fluoride effects in the skeleton (BARTOLUCCI 1912, BAUER 1945, BELANGER et al 1958) Accumulation of osteoid on the trabecular surface makes a quantitative estimation of bone formation by means of the extent of osteoid seams difficult (JOWSEY, SCHENK and REUTTER 1968). For this reason, our estimation of bone turnover is based directly on measurements of the surface covered by osteoblasts, and as far as resorption is concerned, on osteoclast counts The most conspicuous and constant finding is the tremendous increase in the number of osteoblasts towards the end of the

first year, whereby the number of osteoclasts is augmented only to a lesser degree. This difference explains sufficiently the gain in bone substance during our period of observation. It must be emphasized that new osteoid is laid down preferably along neutral surfaces, in contrast to renal osteodystrophy, where these deposits usually cover the scalloped areas originating from a preceeding osteoclastic resorption.

The assumption that the stimulation of osteoblasts and osteoclasts may represent a hyperactivity of the parathyroid glands has been expressed independently by different authors. It was originally incited by the presence of large resorption cavities with fibrous bone marrow in the cortex of long bones of fluoride-exposed man and animals. An increased amount of parathyroid hormone in serum, and electron microscopic evidence of increased cellular activity in the parathyroids of sheep after fluoride application were reported by FACCINI and CARE (1965). NICHOLS, FLANAGAN and WOODS (1965) noticed a striking similarity between the metabolic pattern of florid hyperparathyroidism and that of a patient with radiological evidence of fluorosis. They attributed the marked stimulation of new bone formation in fluorosis to the increased level of parathyroid hormone and postulated a kind of secondary hyperparathyroidism induced by a stabilization and a reduced solubility of the bone salt. Finally, BERNSTEIN and COHEN (1967) discovered 3 cases of slight parathyroid hyperplasia in a group of 30 patients subjected to a long-term fluoride therapy. A coincidence of hyperparathyroidism and positive skeletal balance can only be explained by the assumption of a concurrent inhibition of bone resorption. POSNER et al. (1963) suggested that the substitution of the hydroxyl ion by fluoride stabilizes the apatite crystals and impedes their dissolution. The reduced solubility would also explain the normal serum calcium values observed in most of the clinical studies (RICH and ENSINCK 1961, RICH et al. 1964). FACCINI (1967) reported a higher resistance of fluoride-containing bone against osteoclastic resorption in rabbits supplied with fluoridated water. He admitts, however, that this finding cannot explain the significant increase in circulating parathyroid hormone only one week after fluoride administration (FACCINI 1969), and similar observations of YATES et al. (1964) after intraperitoneal lavage in rats.

Osteoclasts resorbing tetracycline-labelled, fluoride-containing bone are a common finding in our material, where as tunneling resorption of preexisting bone occurs only incidentally (unpublished observation). Therefore, the concept of direct inhibition of bone resorbing cells by fluoride becomes more likely. It is supported by GOLDHABER (1967), who observed an inhibitory effect of fluoride on parathormone induced resorption of mouse calvaria grown in tissue culture. Thus, primary inhibition of enzymatic activity of

bone resorbing cells is supposed to produce a drop in serum calcium, which in turn stimulates the parathyroids. The increased level of PTH stimulates both osteoblastic and osteoclastic activity. As the inhibition of resorbing cells by fluoride continues, bone formation overwhelms osteoclastic bone resorption. This gives rise to a positive skeletal balance and a normal or slightly lowered serum calcium concentration.

Beside the acceleration of bone remodelling and the concomitant inhibition of resorption, the existence of a transient mineralization deficiency is the most prominent histological feature. An osteomalacic effect of fluoride was first suggested by BARTOLUCCI (1912) in fluoride-intoxicated cattle. In man, it was observed in cryolite workers by MOLLER and GUDJONSSON (1932) and ROHOLM (1938). Since fluoride has been used in the treatment of bone diseases, an interference with the mineralization process has been detected regularly (BERNSTEIN and COHEN 1967, BAYLINK and BERNSTEIN 1967, EPKER 1967, JOWSEY, SCHENK and REUTTER 1968). There is, however, not a complete block of mineralization, but rather a delay, as suggested by a diffuse uptake of tetracycline and a persistance of low mineral density in the newly formed bone. In addition, this bone exhibits uncalcified cement lines and poorly mineralized areas around osteocyte lacunae.

The apparent recovery of mineralization after long-term treatment is an unexpected and exciting observation in our material. It is present in all 5 patients which could be observed over a period of more than one year. Before discussing and interpreting this finding, some inherent modifications of the treatment have to be considered:

1 When the existence of a malacic state after six or eight months of treatment became obvious, additional vitamin D was administered periodically in most of the patients.

2 As both clinical and histological examination revealed a distinct improvement of the skeletal disorder and a marked acceleration of bone formation, the daily intake of sodium fluoride was reduced from 50–80 mg to 25–50 mg or, incidentally, completely stopped.

A positive effect of vitamin D on the mineralization process is possible. In the course of our study, additional vitamin D was given regularly to all the subsequent patients. However, vitamin D administration, even from the beginning, could not prevent a transitory surface osteomalacia of about the same extent as in cases without vitamin D. On the other hand, it supported the osteoblastic stimulation to a considerable degree. At the given moment, a closer examination of this interaction is in progress.

The second factor involved in the improvement of osteoid calcification is the reduction in the daily intake of fluoride. Systematic studies on time-dose

relationships in fluoride treated animals and patients are scanty and not conclusive, although ROHOLM (1937) already stated that comparatively small doses resulted in accelerated bone growth and calcification, while large doses produced a mineralization defect. Recovery from abnormal mineralization produced by fluoride in rat incisor dentin has been reported by YAEGER (1966). In the course of our study, the therapeutical success motivated a reduction of the daily intake or a complete cessation of the drug after one or more years. Thus, this factor may well be involved in the apparent recovery from mineralization deficiency in the later periods of observation, especially in the long-term cases discussed in this paper (Fig. 1–4, 10, 11). The fact, that the final stage of severe fluorosis in cryolite workers is almost consistently depicted as osteosclerosis, is another indication for a certain adaptation of the mineralization process. This would imply the elaboration of a more appropriate application schedule with periods of fluoride induced osteoblast stimulation and intermittent recovery periods, both assisted by supplementary vitamin D. This, however, requires a careful further exploration by means of more animal experiments and additional clinical studies.

SUMMARY

This quantitative-histological study deals with structural and turnover changes in 48 serial bone biopsies obtained from 16 osteoporotic patients treated with sodium fluoride. Control biopsies at the beginning of the therapy confirm the diagnosis of senile osteoporosis by the presence of an atrophic trabecular framework and a marked reduction in the number of osteoblasts. The main effects of fluoride administration are as follows:

1. Volumetric density of cancellous bone shows a marked increase, reaching about twice its original value after 80 weeks of treatment. This augmentation is mainly due to an apposition of new bone on the surface of preexisting trabecula.

2. The positive skeletal balance is caused by a tremendous stimulation of bone formation. Thus, the average surface extent of osteoblast layers reaches, after 40 weeks of treatment, a maximum which is 17 times higher than the original value.

3. At the same time, a retardation of osteoid mineralization becomes obvious, its maximum also showing up after 40 weeks. In later periods of treatment, an apparent recovery from this mineralization delay was noted. This may be partly due to a reduction of the daily intake of sodium fluoride, and/or an additional application of vitamin D.

4. However vitamin D, when administered together with fluoride from

the beginning, could not prevent a temporary surface osteomalacia. Its main effect is a marked acceleration of the other effects of fluoride, e.g. the stimulation of osteoblasts.

The questions may arise, whether these results represent a real therapeutic effect, or a more toxic skeletal alteration. We feel, that they at least offer many aspects for further investigation, in order to elaborate a more appropriate application schedule which makes benefit of the powerful stimulation of bone formation and reduces the risks of mineralization delay and other side effects.

ACKNOWLEDGEMENTS

This work is supported by the Swiss National Foundation for scientific research (grant no. 4294) and the Sandoz Foundation for the promotion of medical-biological sciences. The authors wish to thank Miss Y. Litzistorf and Mr. N. Bischof for the excellent technical assistance, as well as Mr. H. Muspach and Miss A. de Lèche for their help in elaborating the diagrams and the manuscript.

REFERENCES

Bartolucci, A.: Casi interessanti di osteite malacia nei bovini. Mod. Zooiat. *23*, 194 (1912).

Bauer, W. M.: Experimental chronic fluoride intoxication. Amer. J. Orthodont. *31*, 700 (1945).

Baylink, D. J., and Bernstein, D. S.: The effects of fluoride therapy in metabolic bone disease. Clin. Orthop. *55*, 51 (1967).

Belanger, L. F., Visek, W. J., Lotz, W. E., and Comar, C. L.: Rachitomimetic effects of fluoride feeding on the skeletal tissues in growing pigs. Amer. J. Path. *34*, 25 (1958).

Bernstein, D., and Cohen, P.: Use of sodium fluoride in the treatment of osteoporosis. J. clin. Endocr. *27*, 197 (1967).

Burkhardt, R.: Technische Verbesserungen und Anwendungsb...

Faccini, A., and Care, A. D.: The effect of sodium fluoride on the ultrastructure of the parathyroid glands of the sheep. Nature (London) *207*, 1399 (1965).

Faccini, J. M.: Inhibition of bone resorption in the rabbit by fluoride. Nature (London) *214*, 1269 (1967).

Faccini, J. M.: Fluoride and bone. Calc. Tiss. Res. *3*, 1 (1969).

Frost, H. M., Villanueva, A. R., and Roth, H.: Halo volume. Henry Ford Hosp. Med. Bull. *8*, 228 (1960).

Goldhaber, P.: The inhibition of bone resorption in tissue culture by nontoxic concentrations of sodium fluoride. Israel. J. med. Sci. *3*, 617 (1967).

JOWSEY, J., KELLY, P. J., RIGGS, B. L., BIANCO, A. J., SCHOLZ, D. A., and GERSHON-COHEN, J.: Quantitative microradiographic studies of normal and osteoporotic bone. J. Bone Jt. Surg. *47*, 785 (1965).

JOWSEY, J., SCHENK, R. K., and REUTTER, F. W.: Some results of the effect of fluoride on bone tissue in osteoporosis. J. clin. Endocrin. *28*, 869 (1968).

MERZ, W. A.: Die Streckenmessung an gerichteten Strukturen im Mikroskop und ihre Anwendung zur Bestimmung von Oberflächen-Volumen-Relationen im Knochengewebe. Mikroskopie *22*, 132 (1967).

MERZ, W. A., and SCHENK, R. K.: Quantitative structural analysis of human cancellous bone. Acta anat. (in press).

MERZ, W. A., and SCHENK, R. K.: A quantitative histological study on bone formation in human cancellous bone. Acta anat. (in press).

NICHOLS, G. Jr., FLANAGAN B., and WOODS, J. F.: Parathyroid influences on bone biosynthetic mechanisms; in GAILLARD, P. J. and TALMAGE, R. V.: The parathyroid glands: ultrastucture, secretion and function, pp. 243 (Univ. Chicago Press, (Chicago 1965).

POSNER, A. S., EVANS, E. D., HARPER, R. A., and ZIPKIN, I.: X-ray diffraction analysis of fluoride on human bone apatite, Arch. Oral Biol. *8*, 549 (1963).

RICH, C., and ENSINCK, J.: Effect of sodium fluoride on calcium metabolism in human beings Nature, Lond. *191*, 184 (1961).

RICH, C., ENSINCK, J., and IVANOVICH, P.: The effects of sodium fluoride on calcium metabolism of subjects with metabolic bone diseases. J. clin. Invest. *43*, 545 (1964).

SCHENK, R. K.: Zur histologischen Verarbeitung von unentkalktem Knochen. Acta anat. *60*, 3 (1965).

SCHENK, R. K., und MERZ, W. A.: Histologisch-morphometrische Untersuchungen über Altersatrophie und senile Osteoporose in der Spongiosa des Beckenkammes. Dtsch. med. Wschr. *94*, 206 (1969).

SCHENK, R. K., MERZ, W. A., and MÜLLER, J.: A quantitative histological study on bone resorption in human cancellous bone. Acta anat. (in press).

SEDLIN, E. D., FROST, H. M., and VILLANUEVA, A. R.: Age changes in resorption in human rib cortex. J. Geront. *18*, 345 (1963).

YAEGER, J. A.: Recovery of rat incisor dentin from abnormal mineralization produced by strontium and fluoride. Anat. Rec. *154*, 661 (1966).

YATES, C., DOTY, S., and TALMAGE, R. V.: Effects of sodium fluoride on calcium homeostasis. Proc. Soc. exp. Biol. *115*, 1103 (1964).

The histological evaluation of bone in fluoride treated osteoporosis

F. KUHLENCORDT, H.-P. KRUSE, L. ECKERMEIER and C. LOZANO-TONKIN

7 patients with osteoporosis were treated with different doses of NaF for varying periods of time. In the following, the results of the histological examination of bone biopsies obtained from the iliac crest and the 7th rib prior to and after therapy are reported. The results of other examinations on these patients, specifically those relating to the dynamics of calcium metabolism, will not be considered here AHRENS, in a separate report, will discuss the excretion of fluoride by these patients (p. 175).

PATIENTS

The group of 7 patients studied consisted of 4 males and 3 females whose ages ranged between 37 and 59 years. 5 patients had primary osteoporosis in a severe form, while in 2 patients secondary osteoporosis in a less severe form was noted Their characteristics are reported in Table 1. The daily dose of sodium fluoride varied between 25 and 150 mg, whereas the actual duration of therapy ranged between 75 and 350 days. Thus, the total amount of NaF administered until the time of the second bone biopsy was between 2 13 and 33 0 g (Table 2) The administration of a different daily dose of NaF was based on a) an attempt to determine in a larger series the optimal amount of fluoride to be given and, b) the necessity to adjust the dose to the individual because of gastrointestinal side effects (nausea, vomiting, diarrhea, heartburn).

METHODS

The bone material was obtained from the iliac crest by needle biopsy and/or by partial resection of the 7th rib. The undecalcified tissue was embedded in methylmetacrylat and ground sections of 50 μ thickness were

Table 1. Listing of patients examined noting age, sex, type of osteoporosis, and important skeletal findings. The evaluation of the stage of osteoporosis was based on clinical, roentgenological and histological criteria (I = mild; II = middle; III = severe).

Case	*Age (years)*	*Sex*	*Type of osteoporosis*	*Skeletal findings*	*Stage of osteoporosis (I–III)*
1 25774/68 (HÖPPNER)	37	♀	primary	Scoliosis of the lower spine	III
2 31265/68 (HEIDEMANN)	38	♂	primary	Kyphoscoliosis, fractures of the roof plate	III
3 17871/69 (GASTEIER)	38	♂	primary	Fractures of the roof plate	II
4 39001/69 (MÜLLER)	48	♂	primary	Scoliosis, wedged vertebrae, thoracic "cod fish" vertebrae, rib fractures	III
5 13307/67 (STÖVER)	58	♀	primary	Kyphoscoliosis, "cod fish" vertebrae, fractures of the roof plate	III
6 7991/69 (DÜTSCHKE)	56	♂	secondary? status post bilateral orchidectomy 1950/51	Kyphosis of thoracic spine, wedged vertebrae, fractures of roof plate	I
7 10687/68 (PRIEWE)	59	♀	secondary, multiple myeloma	No deformities Local bone defects	II

prepared. In addition, thinner sections were made and stained with azur-eosin and by the GOLDNER method. Microsections of 100 μ thickness from the iliac crest and 7th rib were also prepared for quantitative microradiography according to the JOWSEY method. Measurements of the maximal width of the individual osteoid seams of the entire, unstained, 50 μ thick microsections were undertaken. The arithmetical mean of the individual measurements in microns is listed in Table 3. The microradiography was

Table 2. The average daily dose and the total dose of NaF were computed from the duration of NaF therapy and the daily administered dose.

Case	*Total dose NaF (gm)**	*Daily dose (mg)*	*Duration therapy (days)**	*Average daily dose (mg)*
1	2 15	25–50	75	28.7
2	10 90	50–100	159	68.5
3	33.00	100 50	310 40 } 350	94 3
4	20 35	100 50	69 269 } 338	60 2
5	15 00	100–150	132	113 6
6	5 90	50	118	50 0
7	4 55	50	91	50 0

* Based on the time interval between the first and second histological bone examination (first examination prior to onset of therapy)

used qualitatively for determination of the mineralization of the therapeutically induced new bone

Prior to the bone biopsy tetracycline was given twice at standard intervals and the labels were identified under UV light

RESULTS

Biopsies before and after therapy with NaF were performed as shown in Table 3, in order to carry out a comparative examination of the width of the osteoid seams before as well as after therapy

Before analysing the individual cases (Table 3), it should be pointed out that prior to therapy, the width of the osteoid seams in the iliac crest and rib sections showed considerable variation so that a certain amount of reserve must be exercised in comparing the results. The examination of the rib sections in cases 1, 2 and 6 demonstrated generally wider osteoid seams as compared to the sections from the iliac crest in these cases. On the other hand, osteoid seams from the iliac crest as well as from the rib prior to therapy were within or below the physiological range Usually, considerable differences in the width of the seams in the compact as compared to the

Table 1. Listing of patients examined noting age, sex, type of osteoporosis, and important skeletal findings. The evaluation of the stage of osteoporosis was based on clinical, roentgenological and histological criteria (I = mild; II = middle; III = severe).

Case	Age (years)	Sex	Type of osteoporosis	Skeletal findings	Stage of osteoporosis (I–III)
1 25774/68 (HOPPNER)	37	♀	primary	Scoliosis of the lower spine	III
2 31265/68 (HEIDMANN)	38	♂	primary	Kyphoscoliosis, fractures of the roof plate	III
3 17871/69 (GASTNER)	38	♂	primary	Fractures of the roof plate	II
4 39001/69 (MUELLER)	48	♂	primary	Scoliosis, wedged vertebrae, thoracic "cod fish" vertebrae, rib fractures	III
5 13307/67 (STOVER)	58	♀	primary	Kyphoscoliosis, "cod fish" vertebrae, fractures of the roof plate	III
6 7991/69 (DÜTSCHKE)	56	♂	secondary? status post bilateral orchidectomy 1950/51	Kyphosis of thoracic spine, wedged vertebrae, fractures of roof plate	I
7 10687/68 (PRIEWE)	59	♀	secondary, multiple myeloma	No deformities Local bone defects	II

prepared. In addition, thinner sections were made and stained with azur-eosin and by the GOLDNER method. Microsections of 100 μ thickness from the iliac crest and 7th rib were also prepared for quantitative microradiography according to the JOWSEY method. Measurements of the maximal width of the individual osteoid seams of the entire, unstained, 50 μ thick microsections were undertaken. The arithmetical mean of the individual measurements in microns is listed in Table 3. The microradiography was

Table 2 The average daily dose and the total dose of NaF were computed from the duration of NaF therapy and the daily administered dose.

Case	*Total dose NaF (gm)**	*Daily dose (mg)*	*Duration therapy (days)**	*Average daily dose (mg)*
1	2.15	25–50	75	28 7
2	10 90	50–100	159	68.5
3	33 00	100 50	310 40 } 350	94 3
4	20 35	100 50	69 269 } 338	60 2
5	15 00	100–150	132	113.6
6	5 90	50	118	50 0
7	4 55	50	91	50 0

* Based on the time interval between the first and second histological bone examination (first examination prior to onset of therapy).

used qualitatively for determination of the mineralization of the therapeutically induced new bone

Prior to the bone biopsy tetracycline was given twice at standard intervals and the labels were identified under UV light.

RESULTS

Biopsies before and after therapy with NaF were performed as shown in Table 3, in order to carry out a comparative examination of the width of the osteoid seams before as well as after therapy.

Before analysing the individual cases (Table 3), it should be pointed out that prior to therapy, the width of the osteoid seams in the iliac crest and rib sections showed considerable variation so that a certain amount of reserve must be exercised in comparing the results. The examination of the rib sections in cases 1, 2 and 6 demonstrated generally wider osteoid seams as compared to the sections from the iliac crest in these cases. On the other hand, osteoid seams from the iliac crest as well as from the rib prior to therapy were within or below the physiological range. Usually, considerable differences in the width of the seams in the compact as compared to the

Table 3. The table shows the average width of the osteoid seams from the iliac crest and rib cross-sections prior to as well as following therapy with fluoride. The indicated values in μ represent the arithmetical mean of the maximal width of all the osteoid seams present in the histological preparation.

Case	*Iliac crest*		*Rib cross-section*	
	before therapy	*after therapy*	*before therapy*	*after therapy*
1	C: 0 S: 0	CN: 48 μ S: 17 μ	C: 8 μ S: 19 μ	
2	CN: } 4 μ S: }	CN: 20 μ S: 6 μ	C: 0 S: 6 μ	
3	C: 0 S: 4 μ	C: 1 μ S: 8 μ		C: 1 μ S: 15 μ
4	C: 0 S: 0	C: 0 S: 12 μ		C: 15 μ S: 14 μ
5	C: – S: 11 μ			C: 20 μ S: 53 μ
6	C: 0 S: 10 μ	C: 24 μ S: 16 μ	C: } 13 μ S: }	C: 16 μ S: 45 μ
7	CN: 0 S: 8 μ	CN: 16 μ S: 11 μ		

* In the area of the C = Compacta
CN = Spongiosa adjacent to the compacta
S = Spongiosa

cancellous bone were noted. Therefore, the measurements are recorded and considered separately.

Following therapy with NaF (Table 2) all cases demonstrated a widening of the osteoid seams, although to a variable extent (Table 3). In cases 1, 2, 6 and 7 the increase in the width of the osteoid seams in the compact and adjacent cancellous bone was especially distinct, as compared to that seen prior to the onset of therapy. Even the width of the osteoid seams in the cancellous bone was increased in these cases. Following therapy, tremendous new osteoid formation on practically all the inner surfaces of the rib section in case number 5 is striking.

Double tetracycline labels, prior to NaF therapy, were occasionally found in cases 1, 4 and 6, whereas case number 7 was not labelled. Following therapy and repeated labelling, cases 4, 6 and 7 demonstrated isolated, and

Fig 1 A section of a microradiograph of the rib cross-section of case number 5. At this time, NaF therapy amounted to a total of 15 0 gms administered during 132 days Visible is the deficient mineralization in the areas of the periosteocytes of the newly formed bone, leading to a characteristic picture of gross porosity

case number 3 multiple, double labels In case number 2 a second dose of tetracycline was not given prior to the second biopsy.

Microradiographical examinations of the bone sections demonstrated a partial mineralization of the therapy-induced new bone matrix. Mineralization was particularly deficient around the osteocyte lacunae as visualized by the porosity picture (Fig 1)

DISCUSSION

An increase in the width of osteoid seams was noted in all the present cases of osteoporosis who were treated with NaF. Since this effect was quite variable, however, the question can be raised whether a relationship to the dose and duration of the therapy exists Of particular interest in this respect are patients 1, 6, and 7 Despite the smallest total dose of NaF (2.15 g, 5 90 g, 4 55 g) for only short periods of time (days: 75, 118, 91), an extensive effect on the bone was apparent, whereas in case number 3, who received 33 0 g of NaF within 350 days, an only minute effect was noted. This applies to case number 4, as well (338 days, 20 35 g of NaF demonstrating osteoid seam width up to 15 μ) These last two cases, however in other respects, are entirely different from each other since only case number 3 showed multiple double labelling in the second biopsy. Pointing to mineralization of the newly formed matrix in this case which was not the case in patient number 4.

The microradiographic examination suggests that the tissue present within

the NaF induced areas of new bone formation is atypical. Further experimentation is required to determine whether it is possible to eliminate this pathological mineralization of the matrix by combining NaF therapy with vitamin D, and, if necessary, calcium.

To avoid misunderstandings, it should be reiterated that measurements of the bone mass following therapy have not been included in this work. It is therefore not possible to demonstrate an increase in the total bone mass following therapy.

There was no indication, either clinical or histological, to suggest an activation of the parathyroid glands under the NaF therapy.

SUMMARY

The effect of fluoride therapy was tested in 7 patients with primary and secondary osteoporosis. NaF was administered in doses of 2.15 g to 33.0 g within periods of time ranging from 75 to 350 days. The width of the osteoid seams before as well as after therapy was measured using non-decalcified sections from the iliac crest and the 7th rib. All patients demonstrated an increase in the width of the osteoid seams, whereby even with the lowest doses a distinct formation of bone matrix was evident. At the same time, an atypical mineralization of the newly formed bone matrix was noted.

The excretion of fluoride by osteoporotic patients under sodium fluoride therapy

(Preliminary communication)

G. Ahrens

Extensive research has been done on the metabolism of fluoride. From this experimental work a rather clear picture has arisen about the pathways of fluoride in the human and animal body. Fluoride administered orally is absorbed in the gastro-intestinal tract The absorption rate depends upon the solubility of the given substance Fluorides with high solubility will be absorbed up to almost 100%. The portion not absorbed will be excreted with the feces A rigid homeostasis for fluoride is maintained by the body, and even high doses of fluoride will cause only a small and temporary increase of the fluoride concentration in the blood. This rather constant blood level is achieved by means of urinary excretion and bone storage of the excess fluoride. Therefore the difference between fluoride absorption and urinary excretion determines the amount of fluoride stored in the bone.

In connection with the investigations by Kuhlencordt et al. (p. 169) we studied the excretion of fluoride in individuals suffering from osteoporotic diseases Some patients received 50 mg of sodium fluoride in gelatine capsules once a day, while others received 25 mg twice a day.

As of the present date our study includes 13 patients receiving sodium fluoride therapy for periods ranging from one day to 16 months. Following the administration of fluoride, the entire urine was collected over 24 hours in 5 to 7 portions In two cases feces were collected simultaneously The fluoride concentration in urine was determined by a diffusion technique and in the feces the conventional destillation technique was used

Results

Table 1 shows the curves of 8 patients, 4 of whom received fluoride therapy for the first time, and 4 who had been receiving fluoride therapy for at least one month. As can be seen on Table 1 the fluoride concentration in the

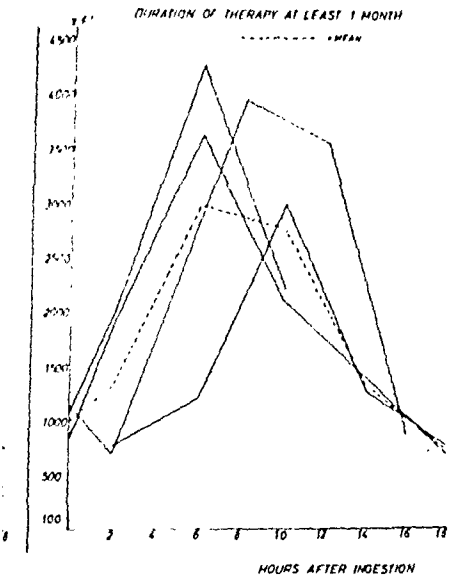


Fig. 1. Daily urinary excretion of fluoride in patients under NaF-therapy (50 mg per day).

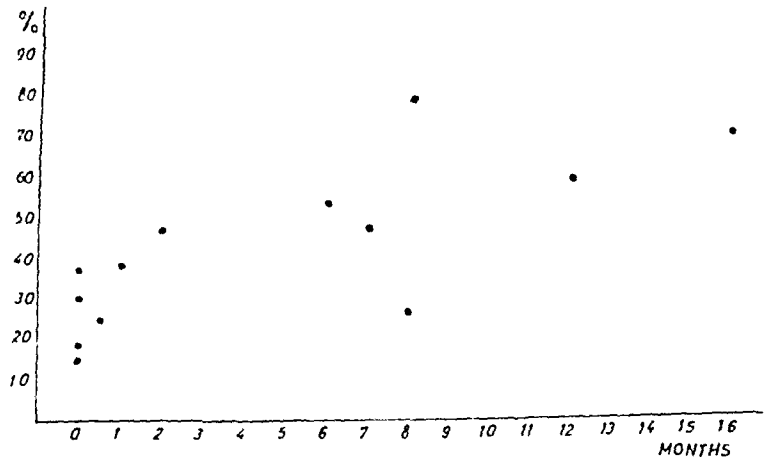


Fig. 2. Percentage of fluoride excreted with the urine during 24 hours in patients with different duration of fluoride therapy (50 mg NaF per day).

urine increased markedly immediately after ingestion of sodium fluoride. We found the highest level 6 hours later. After 24 hours, in most of the cases the urinary fluoride concentration was nearly at the same level as at the beginning of the experiment. In those cases where sodium fluoride was administered for the first time, the excretion curves seemed to take lower courses (see Fig 1 left side) than those following a longer period of fluoride therapy (see Fig. 1)

In order to obtain more information, we calculated the percentage of fluoride excreted during 24 hours in relation to the quantity ingested. In the calculation it was presumed that the excretion in sweat and saliva was negligible. In two cases the excretion in feces was 7.7% and 10% respectively of the ingested 50 mg of sodium fluoride. In the other cases fluoride excretion in the feces was not determined.

In Fig 2 the percentage excreted is plotted against the duration of the therapy. We have an insufficient number of cases to evaluate these results statistically, but the rate of excretion tends to increase slightly with time.

If this tendency is confirmed by further experiments, we must assume that after a certain amount of time a steady state is achieved between ingestion and excretion of fluoride, even where high doses of fluoride are given.

More cases are necessary in order to be able to answer the question of whether the individual variations in the excretion rate can be explained by differences in the severity of the disease

Monitoring of fluoride dosage during treatment of bone disease

J.-P. DUSTIN*

I. INTRODUCTION

Although earlier statements have reported beneficial or adverse effects of fluorides on some tissue or function in man, it seems fair to recognize BLACK and MCKAY's descriptions (3, 23) of mottled enamel in 1916 as the starting point of modern research on fluoride effects. The work is all the more interesting because it led, by the very severity of the enamel hypoplasias observed, to the initial "logical" assumption that mottled teeth would be prone to decay. MCKAY himself verified in 1929 the deficiency of common sense in this case: "My own conviction, prior to these examinations and based on the observations of the past several years, was that mottled enamel was not more liable to decay than normal enamel, but to find it consistently less liable in these communities was a complete surprise." (24.)

The recognition of naturally- and later the development of artificially-fluoridated waters as a preventive factor against dental caries has followed at such a pace, and on so broad a front, that already twenty years ago it had become almost impossible for an individual research worker to keep abreast of the relevant publications. The efforts of Mrs. IRENE CAMPBELL (5) in producing as regularly as anyone could hope the "Fluoride Abstracts", renders an invaluable service to all those interested in most of the biologically significant aspects of fluorides. However, amongst the abundant literature available, much—and certainly the part of immediate interest to the dental professions and to public health workers—has centered on the administration of fluoride in doses well below 5 mg of fluoride-ion per individual, per day. Notwithstanding the extensive epidemiological work carried out in America, the confirmation gathered in many countries all around the world, and the impressive mass of physiological experimentation that bears witness

* Dr DUSTIN is at present seconded from the Free University of Brussels to the World Health Organization.

to the lack of side-effects of fluoride employed at caries-protective dosages, it is well known how controversial the issue of prophylactic administration of fluoride is—even to-day. Just to clarify the position, I would merely repeat (DUSTIN, 1962/63) that, as a public health measure, the administration of fluoride for protection against dental caries is one of the best documented in the history of medicine (12).

What *remains to be decided* is whether such prophylaxis should be a matter for individual initiative, or one to be taken up by communities—national or otherwise—depending on each local organizational pattern. In considering this matter, the World Health Organization has followed its usual procedure: first gathering all relevant information, world-wide, then evaluating it in cooperation with the best experts available, and finally producing an Expert Committee Report for everyone to consider, and eventually to act upon. No. 146 of WHO's Technical Report Series (45) was produced *that way in 1958* Yet the research explosion went on, and—correct as it remains—Report 146 became rapidly out-dated as it did not, by its very *nature and* scope, comprise the diversity of specific data, epidemiological and physiological, necessary to deal adequately *with the pros and* cons of fluoride administration

workers, but also because it appears that bone retention may be much higher (27) in osteopathies than we are led to believe from the physiological data in the literature. Furthermore, the clinical results of fluoride therapy in bone diseases are difficult enough to assess (10, 35) under the best metabolic ward conditions, with the highest cooperation of the patient, so that fluoride therapy of such cases would not appear advisable outside academic and/or research institutions—at least at the present time.

The rationale of fluoride therapy for bone demineralization is derived first of all from the notion that to an osteoporotic patient an induced and controlled degree of osteosclerosis might be of benefit (30, 37). Although encouragement in this direction was provided in 1955 by LEONE et al. (17) showing less osteoporosis but no osteofluorosis in X-rays of paired subjects of corresponding age in high- and low-fluoride communities (8 parts per million of fluoride-ion [ppmF] compared to about 0.5 ppmF), the most convincing observation came from BERNSTEIN et al. (2) in 1966 comparing high (2.4 to 5.8 ppmF) and low-fluoride (0.15 to 0.30 ppmF) areas in North Dakota. Having selected communities of comparable ethnic origin (of German or Scandinavian descent), it was shown that asymptomatic osteoporosis could be detected roentgenologically twice as often in the low-fluoride group of women; vertebral collapse was four times as frequent in women (aged 45 to 65 or more) in the low-fluoride group. In men, X-ray evidence of bone demineralization was more frequent than in the corresponding high-fluoride group—although men showed no significant difference in the incidence of vertebral collapse.

Calcium balance measurement

In the meantime, however, RICH, ENSINCK and IVANOVICH (32, 33) had administered high doses of sodium fluoride (up to 120 mg/day) to osteoporotic cases but only two out of the six patients studied responded by a definite improvement. ROSE (36) attempted analogous fluoride therapy together with high calcium intake: but all four of his cases failed to change their calcium balance from negative to positive values. This ties in with the general failure of high calcium administration to maintain positive calcium balances in adults for more than limited periods of time. Besides, the inherent difficulty of measuring such balances accurately, mainly because they depend on accurate fecal sampling, should inhibit the interpretation of any but the most positive data. ROSE himself stresses the difficulties of calcium balance techniques in his 1967 paper on diagnosis and treatment of osteoporosis, although "it is perhaps the only way at present of finding out in a period of only a few weeks whether or not a therapy is beneficial" (35).

On the other hand VAN WAYJEN (43) showed that anabolic hormone treatment, together with high calcium administration, led to more calcium retention in osteoporosis than either measure taken in isolation

In favour of fluoride therapy is the remarkable study of NEER, ZIPKIN, CARBONE and ROSENBERG (27) where an undisputable positive calcium balance was obtained in a case of multiple myeloma; after some four months of sodium fluoride (3 × 20 mg/day), the balance became positive and stayed so for at least a year. The study involved tracer studies and intercompartment kinetics as may be derived only by advanced studies of the progressive reduction of calcium specific activity with time. it appeared that fluoride "has major effects on both bone accretion and bone resorption, leading to an increase in stable bone calcium" (27). This observation is compatible with those of LUKERT, BOLINGER and MEEK (19), in that it also shows no rapid establishment of a positive calcium balance, but the labile, exchangeable calcium compartment appears to be reduced by the fluoride treatment, by about 15% after one week (average of the 7 cases of LUKERT et al) and about 40% after four months in the NEER et al.'s case. This could be interpreted as a reflection of fluoride fixation in bone as it will first exchange superficially with hydroxyls in apatite, and gradually penetrate the crystalline network (a) by isomorphic recrystallization and (b) by fluoride incorporation in newly formed crystals This view may be supported by the 2.5-fold increase of bone fluoride in the NEER case after four months: X-ray diffraction was not tried to establish whether this was accompanied by enlarged crystallinity in the biopsy. This could not have been an oversight as ZIPKIN (1962) had demonstrated this effect in man (49), and MENCZEL (1964) in the rat (25) both working at the time at the National Institutes of Health in Bethesda, Md , USA It may be guessed, however, that bone deposition may have been too slight in the four months of therapy given to an adult to justify a study that may very well have been inconclusive

A striking feature of the NEER case is its very high bone retention of

ion: about 13% of the fluoride presumably sufficient to start bone fluorosis (approximately 20 g of fluoride-ion [11], the equivalent of 5000 ppmF [42], or even 5000 to 6000 ppmF [22] in fat-free dry bone).

Monitoring fluoride exchanges used to be a difficult enterprise, not only because few clinical laboratories undertake fluoride determinations, but also because the main methods available are reliable only in thoroughly experienced hands. Such is still the case, but the recent introduction of the fluoride electrode may, if accompanied by proper internal calibrations and checked periodically against distillation and chemical procedures, facilitate the access of clinical laboratories to fluoride determinations.

One of the first verifications should be that the intestinal absorption of sodium fluoride takes place with comparable efficiency at therapeutic and at prophylactic dosages (about 93%). This would need to be tested using enteric-coated tablets, as the foreseeable epigastric discomfort associated with the ingestion of 20 mg of sodium fluoride on an empty stomach may modify the absorption quite notably (especially if magnesium or aluminium hydroxide is given for symptomatic relief).

A second check should gauge the kidney functions of the subject as chronic renal sclerosis reduces fluoride elimination: occupational fluorosis has been precipitated by such a condition (MAES, DUFAUX and VANDENBROUCKE [20]). As it has been shown that fluoride clearance is regularly higher than the contemporary chloride clearance, but always below that of inulin or of endogenous creatinine (CARLSON et al. [6]), and as 95% of the plasma fluoride is freely dialysable (CARLSON et al. [7])—the remainder is likely to be linked to plasma protein (CIMASONI [9])—there is no need to postulate a tubular excretion of fluoride. This notion is corroborated by the observation that rabbits (39), dying of uranium intoxication (thus practically without functional proximal tubules) still clear fluoride adequately. Some (12) have postulated on the basis of physical chemical analogy that the hydrated fluoride-ion might passively follow the fate of the water of the glomerular filtrate—although this hypothesis requires testing, it is interesting as it may explain the positive correlation observed in fluoride clearance and water diuresis in the dog (and man) (6). In view of this, a patient with an adequate endogenous creatinine clearance and urea clearance should not run incommensurate risks during adequately monitored fluoride therapy.

The significance of urinary excretion of fluoride should be interpreted differently, depending on whether the subject was consistently taking fluoride for any reason (prophylactic, therapeutic or occupational) for several of the preceding months or years. Such individuals, unless their intake is as high as 10 mg/day or more, usually reach a steady state by which their daily

urinary output equals their average daily intake. This correspondence, originally described by McClure and Kinser (21), has been documented for high-fluoride domestic waters up to a concentration of 8 ppmF by Likins, McClure and Steere (18) and by Largent (16). Whatever the mechanisms at play, this relationship is of great value as an estimation of the overall exposure of individuals to fluoride: i e, it is currently used in industrial examinations of personnel handling fluorides. It should be underlined here that such a steady state can only be achieved by adults exposed to minimal or moderate daily intakes of fluoride: Largent (16) showed that at a 20 mg intake of fluoride-ion (merely three-quarters of the most usually administered daily dose in bone disease) a 30 year old subject had not, after 8 years of exposure, reached a fluoride steady state: he was still storing 8 6 mg of fluoride-ion daily. As stated above, patients under high-fluoride therapy must necessarily be in positive fluoride balance: in view of their known intake, periodical urine collections could be determined for fluoride, and the cumulative retention of fluoride estimated with some accuracy. In prolonged treatment, the clinician could then know when he approaches the fluorotic bone concentrations with minimal discomfort to the patient. This should curb most needs for bone biopsies, except for specific histological reasons Reliance on the well known linear relationship between soluble daily doses ingested and their resultant 50% urinary excretion and 50% retention in the body, even if stepped up to 18 mg fluoride-ion/day, is clearly hazardous when treating bone disease. Let us also remember that growing individuals necessarily escape steady state estimations and adaptation rates to changing fluoride exposures: their bone growth keeps them in positive fluoride-ion balance substantially longer than their adult counterparts (Zipkin et al. [47]).

Numerous observations of impaired kidneys clearing amounts of fluoride currently used in caries prevention should not obscure the fact that this may not be so when larger amounts of fluoride need to be eliminated: Largent (16) describes two cases where patients with clinically de-

itored by his cumulative retention of fluoride, as fluorosis may be feared when the overall retention approaches 20 g. Whether the risk of approaching this limit, or even of exceeding it, is worth taking will of course remain up to the clinician's judgement of the circumstances of each case.

Roentgenology

If we turn from our concern about doing no harm to examine whether our treatment is doing any good, we should, like most authors (i.e. DENT and WATSON [10] and ROSE [35]) rely very little on the patient's relief, as it frequently occurs spontaneously in osteoporosis. Besides, placebos have been shown to give as much relief as the fluoride they represented in some of the observed cases.

X-ray evaluation, particularly of representative bones such as the lumbar vertebrae, the pelvis and the cortex of the radius shaft (37) could be of help, but cases are described where it took two years to notice any change in mineralization. If this is related to the statement—possibly pessimistic—that it will take a 30 to 60% reduction of bone mineral to show on a conventional radiograph, LACHMAN (15), there seems to be room for improvement in the techniques used. Having learned from physicists how difficult it is to obtain a monochromatic beam from conventional tubes, it is naturally difficult to make valid densitometric measurements, especially if allowance is to be made for soft tissues. One way out might be, following NILSSON's example (28), the use of monochromatic isotopic sources, such as Americium 241 (^{241}Am), the main emission of which is a 59.6 KeV X-ray beam, and possibly another analogous source of different hardness such as Iodine 125 (^{125}I) (CAMERON [4]). Differential measurements in transmitted photons either by scintillation or by densitometry of geometrically and photographically identical films should, at least in theory, allow monitoring for the mineral content of bone with an accuracy substantially better than the quoted 30%. SAVILLE (37) has led the way in this direction, achieving 3–4% sensitivity by direct densitometry of ^{241}Am X-ray beams. Efforts to improve the monitoring of the mineral content of bone should be attempted whenever possible, for they could conveniently provide the data on which to decide whether a treatment is effective without the inconveniences of bone biopsies.

As previously mentioned, it can be seen that, together with many others, I do not believe that high dose fluoride therapy is yet ripe for any definite formulation—more experimentation is needed before specific therapeutic procedures may be recommended.

III. OUTLOOK REGARDING MEDICALLY CONTROLLED ADMINISTRATION OF FLUORIDES

A more optimistic aspect is seen on the side of prevention. If, as ROSE (35) suggests, the natural history of osteoporosis consists of an increase in bone mass until about 20 years of age, followed by a slow physiological demineralization, a possible approach may be to enhance the growth of bone less prone to demineralization This could be achieved by a regular administration of moderate amounts (about 10 mg/day) of fluoride to children, adolescents and young adults between the ages of 8 and 25. To start earlier would mean disfiguring tooth mottling, and the amount chosen would allow a positive fluoride balance during much of the skeletal mineralization, as well as attainment of a steady state within weeks or months after skeletal adulthood has been reached. Such medication, which I can only see under adequate medical control, would tend to reproduce the favourable situation of high fluoride North Dakotans, as described by BERNSTEIN et al. (2) Even if the cumulative dosage would amount to approximately 60 g over about 17 years, such individuals would be in as steady a state regarding fluoride as their growth would allow, prudently approximating the condition of growth prevailing in Bartlett (Texas) where the domestic waters used to contain 8 ppmF MCCLURE, MCCANN and LEONE (22) showed by their detailed comparison of two cases that after life-long exposure to 8 ppmF drinking water, their subject's bones contained about eight times as much fluoride as the control who spent his life in a 0 5 ppmF area; the subject's skeleton contained about 10% more calcium and phosphorus, in the usual 2:1 calcium over phosphorus proportion. This study also showed that, even at a fluoride concentration of 5 to 6‰ in dry fat-free bone, the skeleton displayed no signs of fluorosis

Although this case may be extreme, the linear relationship established by ZIPKIN et al (48) between the concentrations of fluoride in drinking water and the corresponding fluoride content of dry fat-free bone would readily allow adaptation to any dosage of the suggested order of magnitude (0.2 to 4 0 ppmF in water in linear relation with 0 5 to 4‰ of fluoride-ion in bone) In effect, at the 4 ppmF level of water fluoridation where this linearity is still maintained, the daily intake of the average adult would be around 10 mg of fluoride-ion.

Under adequate supervision, such an approach might, in ROSE's own words, let "fluoride do for the bones what it has done for the teeth" (35).

And HEGSTED, as he reflected on the relative scarcity of aorta calcifications

in high-fluoride groups compared to low-fluoride groups, might even have added: "... somehow fluoride helps to keep the calcium in the bone and to prevent its deposition in blood vessels", but this "additional pay-off" (13), related to the secondary calcification of atheroma lesions, is still another aspect, well beyond the scope of this presentation.

SUMMARY

Having recalled the solid documentation supporting prophylactic dosage of fluoride to reduce the incidence of dental caries, the case of therapeutic dosage to curb or correct bone demineralization is examined. The effectiveness of high dosage of fluoride in this context is difficult to establish, and this therapy should remain carefully experimental at the present time. Monitoring such medication should involve an assessment of the amount of fluoride retained by the patient, with the assurance of sufficient kidney function. Improved roentgenological techniques could possibly be devised to monitor the mineralization trends without resorting routinely to bone biopsies. The possible role of moderate amounts of fluoride medication between the ages of 8 and 25 is envisaged—as a result of the data presently available—as a plausible preventive measure against physiological ageing osteoporosis*.

REFERENCES

1 BERNSTEIN, D. S., and COHEN, P.: Use of sodium fluoride in the treatment of osteoporosis. J. Clin. Endocr. *27*, 197 (1967).

2 BERNSTEIN, D. S., SADOWSKY, N., HAGSTED, D. M., GURI, C. D., and STARE, F. J.: Prevalence of osteoporosis in high- and low-fluoride areas in North Dakota. J. Amer. Med. Assoc. *198*, 499 (1966).

3 BLACK, G. V. (in collaboration with MCKAY, F. S.): Mottled teeth: an endemic developmental imperfection of the enamel of the teeth heretofore unknown in the literature of dentistry. Dental Cosmos *58*, 129 (1916).

4 CAMERON, J. R., and SORENSON, J.: Measurement of bone mineral in vivo: an improved method. Science *142*, 230 (1963).

5 CAMPBELL, I. R., and WIDNER, E. M.: Annotated bibliography: the occurrence and biological effects of fluorine compounds. Cincinnati, Kettering Laboratory, University of Cincinnati, Ohio, USA (1958 onwards).

6 CARLSON, C. H., ARMSTRONG, W. D., and SINGER, L.: Distribution and excretion of radiofluoride in the human. Proc. Soc. Exptl. Biol. (N.Y.) *104*, 235 (1960).

* This paper reflects the personal views of the author and not necessarily the set policy of the World Health Organization.

7 Carlson, C. H., Armstrong, W. D., and Singer, L.: Distribution, migration and binding of whole blood fluoride evaluated with radiofluoride. Amer. J. Physiol. *199*, 187 (1960).
8 Carlson, C. H., Singer, L., and Armstrong, W. D.: Radiofluoride distribution in tissues of normal and nephrectomized rats. Proc. Soc. Exptl. Biol. Med. *103*, 418 (1960).
9 Cimasoni, G.: The binding of fluoride to albumin as studied by gel filtration. (In press: Caries Research, Publ. S. Karger, Basle.)
10 Dent, C. E., and Watson, L.: Osteoporosis. Postgrad. Med. J., Oct. Suppl. (1966).
11 Dustin, J.-P.: La fluorisation prophylactique comporte-t-elle un risque de fluorose osseuse? Z. f. prophylaktische Med. *3*, 66 (1957).
12 Dustin, J.-P.: Aspects physiologiques de la prophylaxie fluorée de la carie dentaire. In: Advances in fluorine research and dental caries prevention. Editors Hardwick, J. L., Dustin, J.-P., and Held, H.-R. Pergamon, London (1963).
13 Hegsted, D. M.: Nutrition, bone and calcified tissue. J. Amer. Dietetic Assoc. *50*, 105 (1967).
14 Jowsey, J., Schenk, R. K., and Reutter, F. W.: Some results of the effect of
15
16 Largent, E. J.: Fluorosis the health aspects of fluorine compounds. Columbus, Ohio State University Press (1961).
17 Leone, N. C., Stevenson, C. A., Hilbish, T. F., and Sosman, M. C.: A roentgenological study of a human population exposed to high fluoride domestic water. Amer. J. Roentgenol. *74*, 874 (1955).
18 Likins, R. C., McClure, F. J., and Steere, A. C.: Urinary excretion of fluoride following defluoridation of a water supply. Publ. Health Repts. (Washington) *71*, 217 (1956).
19 Lukert, B. P., Rottinger, R. E., and Meek, J. C.: Acute effect of fluoride on ^{45}calcium dynamics in osteoporosis. J. Clin. Endocr. *27*, 828 (1967).
20 Maes, J., Dufaux, J., and Vandenbroucke, J.: Chronische fluor-
21
content of urine in relation to fluorine in drinking waters. Publ. Health Repts. (Washington) *59*, 1575 (1944).
22 McClure, F. J., McCann, H. G., and Leone, N. C.: Excessive fluoride in water and bone chemistry. Comparison of two cases. Publ. Health Repts. (Washington) *73*, 741 (1958).

in high-fluoride groups compared to low-fluoride groups, might even have added: "... somehow fluoride helps to keep the calcium in the bone and to prevent its deposition in blood vessels", but this "additional pay-off" (13), related to the secondary calcification of atheroma lesions, is still another aspect, well beyond the scope of this presentation.

SUMMARY

Having recalled the solid documentation supporting prophylactic dosage of fluoride to reduce the incidence of dental caries, the case of therapeutic dosage to curb or correct bone demineralization is examined. The effectiveness of high dosage of fluoride in this context is difficult to establish, and this therapy should remain carefully experimental at the present time. Monitoring such medication should involve an assessment of the amount of fluoride retained by the patient, with the assurance of sufficient kidney function. Improved roentgenological techniques could possibly be devised to monitor the mineralization trends without resorting routinely to bone biopsies. The possible role of moderate amounts of fluoride medication between the ages of 8 and 25 is envisaged—as a result of the data presently available—as a plausible preventive measure against physiological ageing osteoporosis*.

REFERENCES

1 BERNSTEIN, D. S., and COHEN, P.: Use of sodium fluoride in the treatment of osteoporosis. J. Clin. Endocr. *27*, 197 (1967).

2 BERNSTEIN, D. S., SADOWSKY, N., HAGSTED, D. M., GURI, C. D., and STARE, F. J.: Prevalence of osteoporosis in high- and low-fluoride areas in North Dakota. J. Amer. Med. Assoc. *198*, 499 (1966).

3 BLACK, G. V. (in collaboration with MCKAY, F. S.): Mottled teeth: an endemic developmental imperfection of the enamel of the teeth heretofore unknown in the literature of dentistry. Dental Cosmos *58*, 129 (1916).

4 CAMERON, J. R., and SORENSON, J.: Measurement of bone mineral in vivo: an improved method. Science *142*, 230 (1963).

5 CAMPBELL, I. R., and WIDNER, E. M.: Annotated bibliography: the occurrence and biological effects of fluorine compounds. Cincinnati, Kettering Laboratory, University of Cincinnati, Ohio, USA (1958 onwards).

6 CARLSON, C. H., ARMSTRONG, W. D., and SINGER, L.: Distribution and excretion of radiofluoride in the human. Proc. Soc. Exptl. Biol. (N.Y.) *104*, 235 (1960).

* This paper reflects the personal views of the author and not necessarily the set policy of the World Health Organization.

7 Carlson, C. H., Armstrong, W. D., and Singer, L.: Distribution, migration and binding of whole blood fluoride evaluated with radiofluoride. Amer. J. Physiol *199*, 187 (1960).

8 Carlson, C. H., Singer, L., and Armstrong, W. D.: Radiofluoride distribution in tissues of normal and nephrectomized rats. Proc. Soc. Expti. Biol. Med. *103*, 418 (1960)

9 Cimasoni, G.: The binding of fluoride to albumin as studied by gel filtration. (In press: Caries Research, Publ S. Karger, Basle)

10 Dent, C. E., and Watson, L.: Osteoporosis. Postgrad Med. J., Oct. Suppl *(1966)*

11 Dustin, J.-P. La fluorisation prophylactique comporte-t-elle un *risque de* fluorose osseuse? Z. f. prophylaktische Med. *3*, 66 *(1957)*

12 Dustin, J.-P.: Aspects physiologiques de la prophylaxie fluorée de la carie dentaire. In: Advances in fluorine research and dental caries *prevention.* Editors Hardwick, J. L., Dustin, J.-P., and Held, H.-R. Pergamon, London *(1963)*

13 Hegsted, D. M.: Nutrition, bone and calcified tissue. J. Amer. Dietetic Assoc. *50*, 105 (1967).

14 Jowsey, J., Schenk, R. K., and Reutter, F. W.: Some results of the effect of fluorine on bone tissue in osteoporosis. J. Clin. Endocr. *28*, 869 (1968).

15 Lachman, E. *Osteoporosis: the* potentialities and limitations *of its roentgenological* diagnosis Amer J Roentgenol. *74*, 712 (1955)

16 Largent, E. J. Fluorosis *the health aspects of* fluorine compounds. Columbus, Ohio State University Press (1961).

17 Leone, N. C., Stevenson, C. A., Hilbish, T. F., *and* Sosman, M. C.: A roentgenological study of a human population exposed to high fluoride domestic water Amer J Roentgenol *74*, 874 *(1955)*

18 Likins, R. C., McClure, F. J., and Steere, A. C.: Urinary excretion of fluoride following defluoridation of a water supply Publ. Health Repts. (Washington) *71*, 217 *(1956)*

26 Møller, P. F., and Gudjonsson, S. V.: Massive fluorosis of bones and ligaments. Acta Radiol. Scand. *13*, 269 (1932). Reprinted: Clin. Orthopaedics *55*, 5 (1967).
27 Neer, R. M., Zipkin, I., Carbone, P. P., and Rosenberg, L. E.: Effect of sodium fluoride therapy on calcium metabolism in multiple myeloma. J. Clin. Endocr. *26*, 1059 (1966).
28 Nilsson, B. E.: Post-traumatic osteopenia. A quantitative study of the bone mineral mass in the femur following fracture of the tibia in man using Americium-241 as a photon source. Acta Orthop. Scand. *37*, suppl. 91, 1 (1966).
29 Purves, M. J.: Some effects of administering sodium fluoride to patients with Paget's disease. Lancet *2*, 1188 (1962).
30 Rehfuss, E. C., and Albright, E.: In: Josiah Macy Foundation, 8th Conference on metabolic aspects of convalescence including bone and wound healing (1944), p. 21.
31 Reutter, F. W., and Silbenmann, R.: Die Wirkung von Natriumfluorid bei metabolischen Knochenerkrankungen. Helv. Med. Acta *32*, 493 (1965).
32 Rich, C., and Ensinck, J.: Effect of sodium fluoride on calcium metabolism of human beings. Nature *191*, 184 (1961).
33 Rich, C., Ensinck, J., and Ivanovich, P.: The effects of sodium fluoride on calcium metabolism of subjects with metabolic bone disease. J. Clin. Invest. *43*, 545 (1964).
34 Roholm, K.: Fluorine intoxication: a clinical-hygienic study. H. K. Lewis, London (1937).
35 Rose, G. A.: A critique of modern methods of diagnosis and treatment of osteoporosis. Clin. Orthopaedics *55*, 17 (1967).
36 Rose, G. A.: A study of the treatment of osteoporosis with fluoride therapy and high calcium intake. Proc. Roy. Soc. Med. *58*, 436 (1965).
37 Saville, P. D.: Treatment of postmenopausal osteoporosis. Modern treatment *5*, 571 (1968).
38 Shambaugh, G. E., and Petrovic, A.: Effects of sodium fluoride on bone: application to otosclerosis and other decalcifying bone diseases. J. Amer. Med. Assoc. *211*, 969 (1968).
39 Smith, F. A., Gardner, D. E., and Hodge, H. C.: Arch. Environmental Health *11*, 2 (1955).
40 Srikantia, S. G., and Siddiqui, A. H.: Metabolic studies in skeletal fluorosis. Clin. Sci. *28*, 477 (1965).
41 Steinberg, C. L., Gardner, D. E., Smith, F. A., and Hodge, H. C.: Comparison of rheumatoid (ankylosing) spondylitis and crippling fluorosis. Ann. Rheumatic Diseases *14*, 378 (1955).
42 Steinberg, C. L., Gardner, D. E., Smith, F. A., and Hodge, H. C.: Fluoridation of public water supplies and its relation to musculoskeletal diseases. New Engl. J. Med. *258*, 322 (1958).
43 Van Wayjen, R. G. A.: Long-term metabolic balance studies of the protein and calcium metabolism in normal subjects and patients with osteoporosis. Israel. Med. J. *23*, 29 (1964).
44 Verniory, J. P., Rosenbusch, C. A., and Lambert, A.: La calcithérapie massive dans l'ostéoporose. Essai d'un nouveau dérivé calcique (premiers résultats). Helv. Med. Acta *32*, 487 (1965).

45 World Health Organization Expert Committee on Water Fluoridation (First Report) World Health Org. techn. Rep. Ser. *146* (1958).
46 World Health Organization: Fluoride and human health *(in preparation)*.
47 Zipkin, I., Likins, R C , McClure, F. J , and Steere, A. C.: Urinary fluoride levels associated with use of fluoridated waters Publ Health Repts (Washington) 71, 767 (1956)
48 Zipkin, I., McClure, F. J , Leone, N. C., and Lee, W. A.: Fluorine deposition in human bones after prolonged ingestion of fluoride in drinking water. Publ. Health Repts. (Washington) *73*, 732 (1958).
49 Zipkin, I , Posner, A S , and Eanes, E. D.: The effect of fluoride on the X-ray diffraction pattern of the apatite of human bone Biochem. Biophys Acta *59*, 255 (1962).

Conclusions

G. Peters

Rather than dealing with the somewhat too extensive subject of fluorides in medicine, this symposium was mainly devoted to studies on the effect of medium doses of fluoride on healthy and on sick bone; medium doses being defined as doses far above those used for the prevention of dental caries, but below those inducing crippling fluorosis.

The hope that such medium doses might either prevent or cure osteoporosis or other bone diseases accompanied by demineralization was conceived approximately 15 years ago and was based on several experimental facts and lines of thought.

1. The excellent preventive effect of small doses of fluoride against dental caries which, in the words of one of the speakers in this symposium, "is one of the best documented facts in the history of medicine". The protection against dental caries obtained by fluoride is usually ascribed to the deposition of fluoroapatite cristals instead of the ordinary hydroxyapatite cristals in dental enamel. As shown in one of the papers, this may, however, not be the only mechanism of action: fluoride accumulation and toxic effects on microorganisms usually responsible for dental caries, may contribute to some extent. Furthermore, dental enamel is a tissue very different from bone.

2. Several statistical studies appeared to show that osteoporosis occurs less frequently in regions with a high water fluoride content than in those where the inhabitant consume little fluoride. None of these statistical studies was, however, sufficiently extensive to be entirely convincing.

3. Advanced fluorosis is generally characterized by a certain amount of osteosclerosis. Again, in the words of one of the speakers of this symposium, this fact naturally leads to the idea that "to an osteoporotic patient an induced and controlled degree of osteosclerosis might be of benefit".

The papers presented in this monograph cast considerable doubt on these three basic assumptions. They clearly show that fluoride treatment of osteoporosis and other demineralizing bone disease is still a highly experimental measure and has never yet induced clearly proved therapeutic results, without simultaneously inducing some degree of a priori undesirable bone fluo-

rosis. It appears clearly from the conclusions of these important experimental and clinical contributions, that dosage schedules and additional medications other than those used until now must be found and studied, if fluoride therapy is to acquire a definite place in the treatment of bone diseases.

In normal and in diseased bone of experimental animals and of man, medium doses of fluoride induce increased formation as well as increased resorption of bone. The term "medium doses" refers to much higher doses in rats that in man, the factors responsible for this difference in species sensitivity being unknown. Enhancement of bone formation always prevails over enhancement of reabsorption: the total mass of the skeleton consistently increases. Whether this increase gives a mechanical advantage to the patient, however, appears doubtful. While some experimental evidence points to a greater resistance to fracture of the bones of animals treated with low doses of fluoride (after treatment with higher doses the resistance to breakage was equal to or lower than in untreated animals), the clinical evidence, hitherto, does not support the assumption that moderate bone fluorosis confers greater mechanical strength on the skeleton: a few cases of spontaneous fractures in patients treated with high doses and in subjects exposed to industrial fluorosis may argue to the contrary. The additional bone formed under the influence of medium doses of fluoride differs from normal bone by its low rate of mineralization, by its irregular structure, and by its elective distribution in several parts of the skeleton which unfortunately are not those in greatest need of new bone for increasing mechanical strength of the skeleton. Exostoses occurred in a few patients treated with "therapeutic" doses of fluoride. Some of the anomalies may be due to toxic actions of fluorides on osteocytes which may be exposed to higher concentrations than the interlacunar bone.

From all the histological studies presented, it is quite clear that a simple increase in mechanical strength of bone by replacing hydroxyapatite cristals by fluoroapatite, without considerably altering the histological structure of the bone, has not been obtained with medium dose of fluoride It is doubtful, whether improvements in dosage forms will allow to obtain it. The mechanism by which medium doses of fluoride enhance bone formation as well as bone resorption is not known at present. Several hypotheses are extensively discussed by the contributors to this monograph. In patients treated with these doses the renal excretion of calcium consistently decreases; this decrease may not be secondary to increased skeletal deposition of the cation, but may be a primary renal effect and should be studied in greater detail. If the decreased renal excretion of calcium was a consequence of increased skeletal deposition, there should be some degree of hypocalcemia, unless

the renal excretion of calcium is supposed to be under the control of an yet unknown skeleto-renal mediator. A primary renal retention of calciu could lead to increased skeletal apposition, without necessarily induci hypercalcemia, since the blood calcium level is kept very constant by interaction of parathyroid hormone and calcitonine.

While a large number of data argue for some, as yet not very well define role of hypersecretion or parathyroid hormone in the bone effects of flu ride therapy, no data on the influence of fluoride on the secretion or o blood levels of calcitonine appear to be available at present. Hyperpa thyroidism could well account for some of the bone effects of fluoride, a its presence in fluoride treated animals or man appears well established Why this type of hyperparathyroidism, as opposed to all other primary and secondary types of this dysfunction, is not accompanied by hyper calcemia remains to be explained. Though fluoroapatites are less sol than hydroxyapatite and, therefore, less ready to give up calcium to t extracellular fluid and to the blood stream, it appears highly unlikely that, in these patients, the mobilization of calcium from the skeleton could be impaired to such a degree as to prevent hypercalcemia. Again, in fluoride treated patients or animals the compensatory secretion of calcitonine may differ from that observed in normal animals.

An optimistic note on the possibilities of fluoride treatment of demineralizing bone disease appears in two contributions of this monograph describing patients treated with fluoride for more than one year. In such patients who were, however, given lower doses of fluoride in the second than in the first year, and were furthermore given additional vitamin D, the newly formed bone, during the second year, assumed the character of normal bone in histological aspect as well as in regard to mineralization. It is, however, not clear, at present, whether such a desirable change will occur consistently and whether it is due to prolonged treatment, lower doses, or additional vitamin D. In regard to normalization of bone structure, observations in patients with chronic osteofluorosis point to the possibility that the effects of long term exposition to higher doses of fluoride may differ from those of short term treatment.

The idea to use moderate amounts of fluoride for the prevention of osteoporosis by treating adolescents and young adults between the age of 8 and 25, defended by one of the contributors to this symposium, in view of the facts discussed above, appears premature, even if it were possible, at present, to decide which individuals should be exposed to this preventive measure.

The contributions to this symposium certainly mark a milestone in the study of the use of fluoride in therapeutics and of the chronic pharmacology of this anion.